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UNITED STATES DEPARTMENT OF AGRICULTURE, WASHINGTON, D.C.

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PYTHIUM ROOT ROT OF SUGARCANE

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INTRODUCTION

The general decline in yields and repeated crop failures that resulted in virtual bankruptcy of the Louisiana sugar industry during the period from 1923 to 1926 were due mainly to combined damage from three major diseases—mosaic, red rot, and the so-called root disease, or root rot. To all of these the old noble varieties of sugarcane (*Saccharum officinarum* L.) were very susceptible. While the timely introduction of the vigorous, mosaic-tolerant P. O. J. hybrids (*S. officinarum* × *S. barberi* Jeswiet) rapidly restored the industry, certain of these varieties, after a period of 5 to 7 years, in turn began to suffer a serious decline in yield due to red rot (*Colletotrichum falcatum* Went) and apparently also to root rot caused by *Pythium arrhenomanes* Drechsler. Fortunately, there were already available to take their place two new introductions, Co. 281 and Co. 290, and several C. P.[3] seedlings produced at Canal Point, Fla., under the

[1] Submitted for publication May 18, 1938.
[2] The writers are grateful to Charles Cassidy and James E. Taylor for assistance in the greenhouse and laboratory studies.
[3] C. P.=Canal Point.

coordinated sugarcane breeding and testing program of the United States Department of Agriculture, in cooperation with the Louisiana Agricultural Experiment Station and the American Sugar Cane League.

Yields per acre have again risen despite a considerable degree of susceptibility toward root rot of some of these varieties. This latter fact, considered in relation to the previous experience with the P. O. J. varieties, has emphasized the need for fundamental knowledge of root rot as a necessary basis for determining resistance and securing and maintaining further yield improvement. This bulletin presents a general summary of investigations carried on intermittently since 1924.

Previous reports (*74*, *79*)[4] have indicated that the most important root disease of sugarcane in Louisiana is of the nature of a true root rot produced by *Pythium arrhenomanes*. The diversity of strains and evidence of physiologic specialization in this species were emphasized, and more recently (*80*) the influence of certain host-conditioning factors that may be associated with poor drainage of the heavy clay soils has been reported. Major attention, therefore, has been given this species of *Pythium*, although some studies on other root-rotting species, together with a review of literature, are also summarized in this bulletin.

HISTORY AND GEOGRAPHIC DISTRIBUTION

EARLY RECORDS

The expressions "root disease" and "root rot" have been used more or less synonymously and are found in the literature of practically every sugarcane-producing country. In the earlier popular writings apparently any adverse condition, whether of soil, weather, or insect origin, that resulted in stunted growth or premature dying of the cane, not obviously due to above-ground diseases, might be attributed by the planters to root disease. The practically world-wide failure of the Otaheite, or Bourbon, cane (*30*) beginning in the latter half of the last century was also ascribed to root disease, although in the British West Indies, at least according to Nowell (*67*), the primary cause of the failure was probably red rot. Thus, as also pointed out by Earle (*34*, *35*) and other students of the subject, many of these early accounts were complicated by the presence of other diseases which have since been identified.

The failure of the Otaheite cane in Hawaii (where it is called Lahaina), which was apparently less sudden than in the West Indies, has been definitely traced to root troubles, originally termed "Lahaina disease" and more recently designated "root rot" or "growth failure" (*7*). The well-documented history of the disease in Hawaii (*25*, *61*) suggests that the gradual decline, so-called degeneration or running out, of varieties reported in the early literature of other countries may indeed have been due, in part at least, to specific root diseases.

In Louisiana the old Creole cane, the first variety brought to the New World, was said to have shown signs of failure before it was

[4] Italic numbers in parentheses refer to Literature Cited, p. 90.

finally replaced by less delicate sorts. It was brought to Louisiana in 1751, and, together with the Otaheite (introduced in 1797), remained a commercial variety until after 1825, when both were gradually replaced by the Ribbon and Purple (Cheribon) canes. Various letters and agricultural reports to the United States Patent Office during the period 1847–55 refer to degeneration or failure of sugarcane in Louisiana. The situation apparently became so alarming as to call for a joint resolution of the Louisiana Legislature in 1848 (*43*), quoted in part as follows:

Whereas, the culture of the sugar cane is one of the chief resources of the wealth and prosperity of this state; and whereas, it is apprehended by many that the Ribbon cane, the most valuable variety now cultivated in the state, may eventually become deteriorated as the Creole cane has already been, by constant reproduction without any change of soil or climate; therefore be it resolved, * * *

to the effect that the State's representatives in Congress request the national administration to import additional varieties from foreign countries.

Discussing the "terrible year of 1854," D. J. B. (*15*) (presumably D. J. Benjamin) does not credit the theory of degeneration per se, but attributes the general deterioration of the cane to it having become feeble or diseased, and recommends crop rotation, borer-free planting material, and manuring.

While root diseases are not specifically mentioned in these early reports, nor subsequently by Stubbs and Purse (*89*), the failure of ratoon crops and so-called degeneration of the Creole and Otaheite varieties suggest the presence and increasing activity of such troubles as the canelands became older and more compacted. The descriptions give no suggestion of red rot or other present-day diseases, except possibly black rot (*Ceratostomella adiposum* (Butl.) Sartoris).

Apprehensions over the Ribbon cane proved to be unfounded, for, as is well known, it and the Louisiana Purple were the mainstay of the industry during the following three-quarters of a century, or until their partial substitution by D–74. The final collapse of all three occurred in the early 1920's from a combination of mosaic, red rot, and root disease, as mentioned on page 1.

ASSOCIATION OF ROOT-ROTTING ORGANISMS

The identification and study of possible causal organisms of root diseases began in Java, where these troubles were at first confused with the effects of the sereh disease. Following the sereh epidemic of the early eighties and its indirect control by "rejuvenation" in mountain nurseries, root diseases became clearly differentiated and, in spite of discontinuance of ratoon crops, came to be regarded as the most serious menace with which the industry had to deal. In the first publication on sereh in 1885, Treub (*94*) reports finding in the smaller roots of diseased plants, in addition to nematodes, a fungus which he considered should "most probably" be referred to the genus *Pythium*. Investigations by Tschirch (*95*), in 1891, and Wakker (*100*), in 1896, disclaimed any direct connection of this fungus with sereh, and indicated that it was not a *Pythium* but should more properly be classified under the endotrophic mycorrhiza of Frank (*46*). Failing to find fructification or to isolate it in pure culture,

Wakker designates it simply as "root fungus No. 1." He was inclined to consider it a parasite, however, causing but little harm.

During the period 1895–1904, when the long-cultivated Black Cheribon (Louisiana Purple), Striped Preanger (Louisiana Ribbon), Batjan, Loethers, Fiji, White Manila, and other susceptible varieties were grown in Java, hundreds of pages were published on the subject of root diseases. In 1895 Wakker (*99*) described a rhizome or basal stem (dongkellan) disease of nursery plants and attributed both this and the more common "dying off" of advanced, or mature, fields to the activity of a toadstool which he named *Marasmius sacchari* n. sp. However, it was soon discovered that this fungus was but rarely associated with the dying-off symptom which came to be considered the true root rot (Kobus (*58*) and Raciborski (*70*)). Following 1922 root rot again received attention in Java, when the susceptible E. K. 28 was widely grown. While extensive studies were reported on the influence of soil type, composition, fertilization, drainage, and field practices, no detailed investigations of the immediate cause or etiology of root rot have apparently been undertaken.

In the West Indies, the Guianas, and Hawaii root disease of the Bourbon and other susceptible varieties was at first, without much investigation, quite generally attributed to *Marasmius sacchari* Wakker, following the identification of this fungus in 1903 by Howard (*54*) in Barbados. In Louisiana, Fulton (*47*) assigned as the cause the closely similar species *M. plicatus* Wakker, which is now identified as *M. stenophyllus* Mont. Detailed descriptions of root disease and its history in these regions were also given in 1917 by Johnston and Stevenson (*57*) and more recently by Earle (*35*) and Nowell (*67*). While *Marasmius* was found quite generally associated with advanced stages of the trouble and under certain conditions, such as those described by Nowell, was so extensively developed as to cause obvious damage, inoculation experiments by Matz (*66*) and others indicated at least that it could not be considered an active parasite of the roots. It has, therefore, come to be regarded mainly as a basal stem invader of already weakened plants and, as earlier concluded in Java, to play no important primary role in the general root-rot problem.

Carpenter (*23*), who undertook an investigation of the failure of the Lahaina variety in Hawaii, was apparently the first to establish a species of *Pythium* as a causative agent of root rot. This species was first ((*24*), 1921) identified by him as *Pythium butleri* Subr., later ((*25*) 1928) as *P. aphanidermatum* (Edson) Fitzpatrick, and more recently ((*28*), 1934), following taxonomic comparisons by the present writers (*79*), as *P. graminicolum* Subr., which Carpenter considered synonymous with *P. arrhenomanes*. Drechsler (*33*) has recently shown that the two species are distinct and that the cane fungus should be designated *P. arrhenomanes*. Its constant association with Lahaina failure and less severe cases of root rot in more resistant varieties and its ability to destroy roots, particularly under conditions less favorable for its host, were experimentally demonstrated by Carpenter and published in a series of papers from the Hawaiian Sugar Planters' Experiment Station.

An unidentified species of *Pythium* and several strains of *Rhizoctonia* capable of rotting cane roots were reported in 1920 by Matz (*66*) in Puerto Rico. He apparently considered the latter fungus more important as a root parasite. Bourne (*16*), working at the

same time in Barbados, isolated and obtained successful infections with *Rhizoctonia solani* Kühn and *R. palida* Matz, one or other of which he believed was responsible for root disease in that country. After transferring to Puerto Rico, Bourne (*17*) published in 1924 a series of microphotographs of a *Pythium* found in cane roots there that he suggested showed morphological similarity to the Pythium-like fungus figures by Carpenter (*24*) in Hawaii. While these photographs are not particularly clear as to detail, their general appearance indicates considerable likeness to the Hawaiian fungus, or what is now known as *Pythium arrhenomanes*.

In Louisiana, Edgerton and his coworkers (*37*) at first likewise emphasized *Rhizoctonia* as a causative agent in cane root rot. However, more intensive surveys of fungi in rotting roots and inoculation experiments published in 1927 (*38*) and 1929 (*40*) showed that species of *Pythium* were the most abundant organisms present in all sections of the Sugar Belt and that one in particular displayed unusual virulence toward cane roots. In a later report (*60, p. 107*) Tims stated this one should be considered *Pythium arrhenomanes*, which identification is in agreement with studies of independently isolated and tested cultures from Louisiana by the senior writer (*74*). The influence of environmental and other factors on the severity of the disease and the relative resistance of varieties have been reported by Flor (*44*, *45*) and others at the Louisiana station.

In 1930 Roldan (*83*) reported a *Pythium* root rot of maize and sugarcane in the Philippine Islands, and in a more detailed paper in 1932 (*84*) definitely identified and described the fungus as a new variety, *Pythium arrhenomanes* var. *philippinensis*. The latter paper, unfortunately, was overlooked in the writers' recent variability studies and taxonomic survey of the species (*79*).

The brief known history of *Pythium arrhenomanes* in relation to root rots of maize and cereals [5] has been outlined in an earlier paper by the writers (*79*) and more recently by Carpenter (*28*). It has thus far been definitely identified only from Hawaii, the Philippine Islands, Mauritius, Canada, and the United States. Etiological studies of sugarcane root rot in the many other countries reporting root diseases will doubtless greatly extend the known geographic distribution of *P. arrhenomanes*.

Of the many other fungi that have been reported in connection with root diseases of sugarcane, only the most frequently mentioned ones, viz, species of *Marasmius* and *Rhizoctonia*, have been included in this brief literature review. The investigations of earlier workers leave little doubt that with a very susceptible variety, such as the Otaheite, and under certain unfavorable conditions for growth, these fungi can cause appreciable damage to the cane plant. With further study it is probable that still other fungi may be found to cause root diseases, but it is significant that in the two regions (Hawaii and the continental United States) where most intensive investigations have been conducted, *Pythium arrhenomanes* has been incriminated as the principal agent of root rot.

Preliminary reports in 1924, 1926, and 1929 by Rands (*71*, *72*, *74*) called attention to the extensive root destruction by minute soil ani-

[5] Since completion of the manuscript of this bulletin a paper by Elliott and others (*42*) has appeared, ascribing this fungus as the cause of a serious root rot of milo in the southern Great Plains.

mals which often preceded and greatly accentuated root rot of the old varieties in Louisiana. Laceration of root tips and pruning of fine rootlets by the numerous snails, centipedes, springtails, and other minute soil fauna in the heavier lands has since been studied by Edgerton and Tims (*38*) and by entomologists (*55*, *88*). The damage, both direct and indirect, is apparently much less on the more vigorous new varieties and also more easily differentiated from independent root rot caused by *Pythium arrhenomanes*.

ECONOMIC IMPORTANCE

Economic losses and disruption of the sugar industry in the past in many countries, attributed to root diseases, have been mentioned in the foregoing historical account. Since in many instances the precise nature and cause of the trouble (if, indeed, it was solely a root disease) remain unknown, there is little point in recounting actual loss records.

In Louisiana all investigators (*37*, *38*, *72*) agree that root disease was one of the major factors responsible for the failure of the old varieties. No attempt has been made to unravel and individually evaluate the interrelated factors responsible for this complex. It was obviously the final expression of root rot complicated by the serious effect of red rot, soil-fauna damage, and probably mosaic infection.[6] However, root disease and red rot, uncomplicated by mosaic, were apparently jointly responsible for the major decline in yields that took place prior to the introduction of mosaic, this latter disease being effective only in the final collapse of the industry (1923 to 1926). The downward trend of production before and after the spread of mosaic is illustrated in figure 1, which gives the Louisiana State average per acre yields of sugarcane used for sugar during the past half century.

For the first 20 years of this period (1888–1907) an average yield of 19.6 tons of cane per acre was obtained. The area of many plantations was doubled, so that much of the crop was grown on recently cleared or at least fairly new land. In 1908 red rot was noted infecting the seed cane, and root disease was causing widespread damage (*36*, *47*). Yields for the decade 1910 to 1920 declined to 15.1 tons, or 23 percent below the earlier level. Mosaic was first recognized along the Mississippi River in 1919, and in a few years it spread westward throughout the sugar district. Yields for the 5-year period 1923–27 further declined and reached the disastrously low level of 10.5 tons, or an additional 30-percent reduction. During this time mosaic had attained nearly 100-percent infection. With general adoption of disease-tolerating varieties yields have gradually risen, and for the last 2 years (1936–37) they have exceeded the earlier long-period level by approximately 7 percent.

It is not to be concluded, of course, that all of this decline in yield nor even the difference between successive years was due solely to disease. Economic factors, such as the price of sugar, exerted at least an indirect effect on many occasions. McDonald (*62*) and Edgerton and Tims (*38*) have shown that the low yields of certain

[6] In a publication on mosaic as long ago as 1919 Brandes (*18*) emphasized that on account of the practical difficulties in the way of control measures, root rot was to be regarded as a far more serious problem for the Louisiana cane planter than mosaic.

years were definitely correlated with subnormal temperatures and high rainfall during winter and spring, and it is well known that most of the extremes were due largely to unusual conditions, such, for example, as the Mississippi River flood of 1912, the spring drought and September hurricane of 1915, the cold rainy spring of 1919, the prolonged drought of 1924, and the severe borer damage to seed cane combined with a late, wet spring in 1926. Conversely, unusually favorable weather conditions in 1904, 1921, and 1929 were undoubtedly responsible for the peak production of those years.

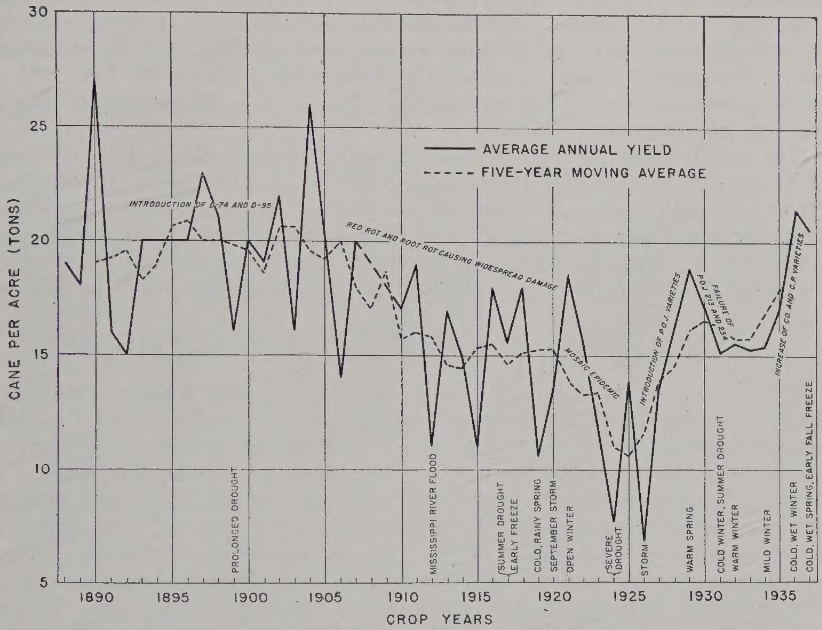


FIGURE 1.—Fifty years of sugarcane production in Louisiana. State average yields of cane per acre used for manufacture of sugar are shown in relation to root rot and other disease prevalence, varietal introductions, and certain extreme weather conditions. Yield data from McDonald (*62*) and reports of the Division of Crop Estimates, United States Department of Agriculture. Records are missing for 1908 and 1909, and that for 1937 (20.5 tons) is the preliminary estimate of December 21, 1937, before completion of the harvesting season.

However, these wide fluctuations have not obscured the general trend of the 5-year average curve in figure 1, which emphasizes the fact that the influence of such unusual conditions was merely superimposed upon a crop the cultivation of which had already become uncertain and even hazardous because of disease. As previously pointed out (*72*), one effect of the diseases was to make the crop more sensitive toward unfavorable weather.

Conservative estimates (*69*) have placed the total financial loss to the sugar industry of Louisiana during the period of low yields (from about 1920 to 1927) at $150,000,000.

In spite of the general adoption of more tolerant hybrid canes, root rot has continued to exert a depressing influence on yields because, as shown later, most of these varieties are in reality moderately sus-

ceptible and are sometimes seriously damaged. The insidious nature of the disease and the great influence of low temperatures, poor drainage, and other unfavorable soil conditions on its severity render unreliable separate estimates of damage from root rot alone. That such is considerable is indicated by comparison of "tolerant" varieties with highly resistant ones under similar field conditions. Co. 281 and Co. 290, respectively, are representatives of these two susceptibility classes. While the usual differences between these two canes in tonnage yields are doubtless due to many factors in addition to possible root rot, unusual differences in maturity are often directly traceable to this cause. Thus, Co. 281, a midseason variety, may be so retarded by root rot during a cool, wet spring that its analyses after November 1 are consistently lower than those of Co. 290, a late-season variety. This represents a loss of 5 to 30 pounds of sugar per ton of cane.

The cultivation of highly resistant varieties, such as Co. 290 and C. P. 29/116, practically eliminates root rot as a factor in production. Unfortunately, these are both late-maturing canes and can be utilized only to a limited extent. However, the progeny studies later considered (pp. 72–74) offer considerable promise of securing varieties combining early maturity with equally high resistance to this disease.

SYMPTOMS AND SEASONAL DEVELOPMENT

Pythium root rot is easily recognized on young plants, carefully dug up in the field, by a flabby, water-soaked condition of the soft terminal portions of the roots, which include, of course, the growing points and water- and food-absorbing part of the root system. A noticeable deficiency of secondary roots and still finer branches or rootlets are indicative of the activity of root rot even though no flabby root ends are found at the time of observation. Roots killed by pythium are invaded immediately by soil fungi and bacteria, and shrivel up or disappear quickly under usual field conditions. In the latter case, only scars, representing their place of attachment to the larger root, remain. On the somewhat older and more resistant parts of the larger roots may be seen reddish, brownish, or black-colored areas, or the whole cortex may have become discolored, without, however, causing serious injury to the central water-conducting cylinder, or stele. This condition should not be confused with the natural darkening and shrinkage of the cortex of old roots, which are normal processes in the development and maturity of all healthy roots. Both tip rotting and cortex lesions may be fairly common on the root systems of vigorously growing and healthy-appearing plants and cause no appreciable damage. The amount of damage depends partly at least upon the age of the plant, whether or not the infection is general, and environmental factors are favorable, the combined effect which is usually detected by critical inspection of the above-ground condition of the field.

ON VERY SUSCEPTIBLE VARIETIES

Gappy stands of plant cane resulting from delayed or deficient germination of the seed pieces because of root destruction are common, although not specific signs of pythium root rot. When the

variety is also very susceptible to red rot (of the planted seed piece), the combined action of the two diseases may result in extensive stand failure, as happened repeatedly with the old varieties formerly grown in Louisiana. The enforced dormancy of the seed cane from rotting of the temporary roots, whose sprouting usually precedes that of the eyes or buds, and the reduced physiological activity of the nodes apparently permit more rapid spread of red rot throughout the seed-stalk. Thus, apparently because of deficient up-take of moisture and nutrients, sprouting of the eyes and emergence of the young plants are prevented or long delayed. Even after coming up, the young plants, with as yet few to no roots of their own, are left partially stranded, so to speak, for moisture and food. Intervention of dry

FIGURE 2.—Severe root rot area (in foreground) of the very susceptible D–74 variety coinciding with an irregular area of silty clay that grades off in the rear to Yazoo very fine sandy loam bearing a healthier crop. Mississippi River bottom field. Photographed May 29, 1925.

weather during this critical period, though preventing further root rot, brings on yellowing of the leaves and death of large numbers of plants. The latter, in 1925, was particularly common in fields of D–74 and Louisiana Purple on "mixed" and heavy soils where both root rot and red rot are always more severe.

Figure 2 illustrates the condition of D–74 plant cane on a Mississippi bottom field near Reserve, La., on May 29, 1925. The margin of a large mixed-soil area in the foreground with its gappy stand of yellowed, wilting plants grades off at the rear into light sandy soil bearing a healthier crop. Representative plants from the two situations (in another field) are shown in figure 3. The outside, less affected plants have more functioning seed-piece roots which have enabled them to put out roots of their own and become established; whereas, the plant in the center, from less favorable soil, has lost both

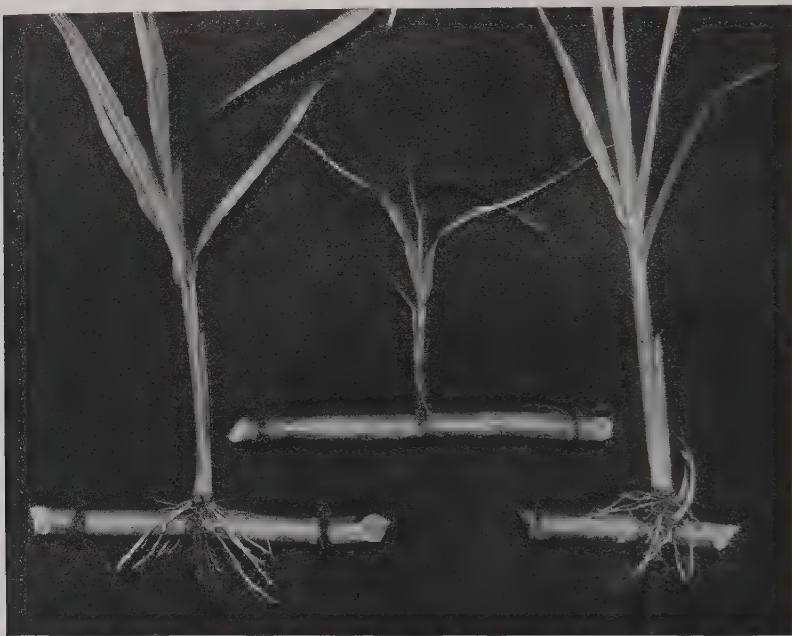
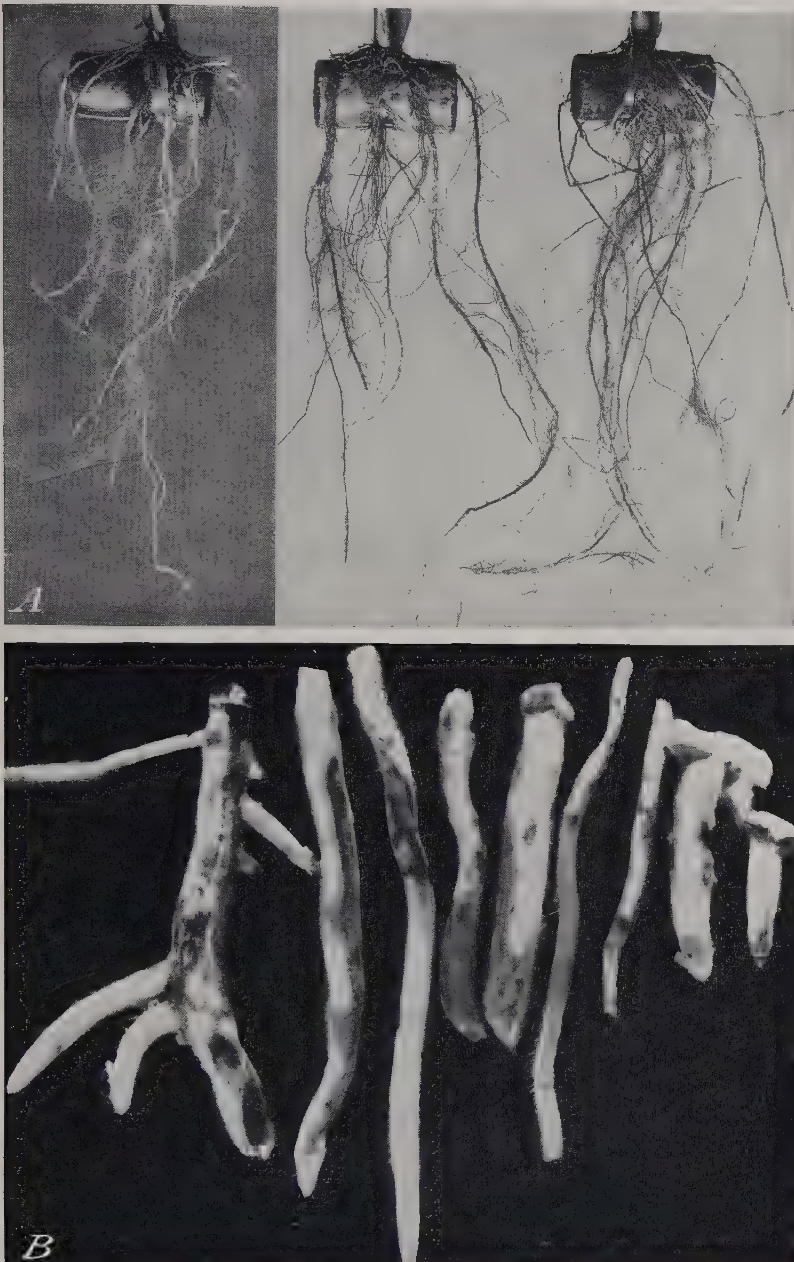
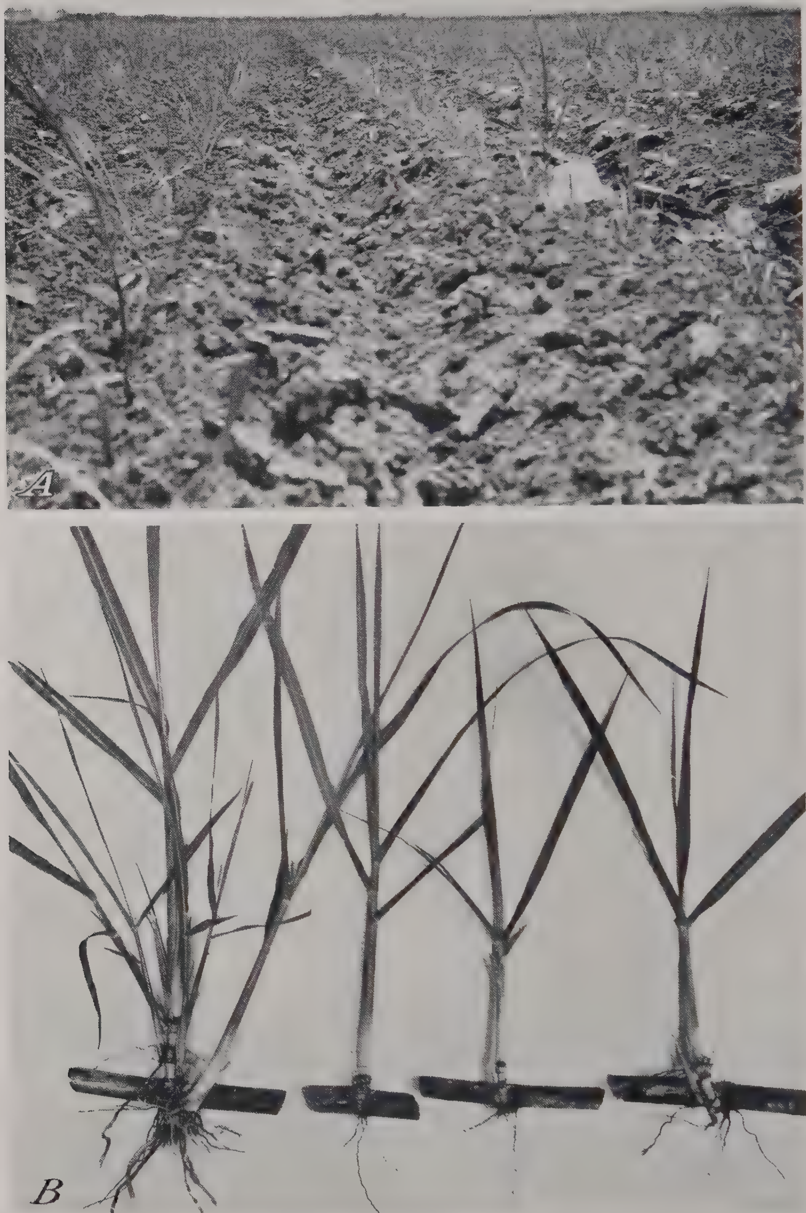


FIGURE 3.—Representative plants of the D-74 variety from two soil types in another field, showing differences in severity of root rot similar to figure 2. The center much stunted, yellowish plant, from the heavier soil, is wholly devoid of functioning roots and has remained largely dependent upon the seed piece (still free from red rot) for its moisture supply. The outside plants, from the lighter soil, while much retarded by root rot, are becoming established on their own roots.

FIGURE 4.—Root-rotted plants of the Louisiana Striped variety attacked by basal stem rot caused by *Marasmius stenophyllus*. The plant at the left was typical of many others in the same fall-planted field, but apparently had not come up until spring when conditions were more favorable for the establishment of a root system. Photographed June 5, 1925.



A, Rootlet rot of the very susceptible D–74 variety caused by *Pythium arrhenomanes* under a midsummer constant soil temperature of 30° C. (86° F.). Uninoculated control at left. Plants grown in the greenhouse in steamed soil inoculated with the fungus, and photographed 44 days after planting. (Photographed by J. F. Brewer.) *B*, Initial shoot roots from a single young plant of the P. O. J. 213 variety showing combined damage from minute soil fauna and root rot. Mildly parasitic species of *Pythium* and other fungi were usually isolated from such material.



Severe damage from root rot to the moderately susceptible C. P. 28/19 variety on heavy clay soil, revealed during a dry spell following a cold, wet spring: *A*, Most of the plants have yellowed and tightly rolled leaves, which form a striking contrast to the occasional dark green and normally developed plants (shown near the hat), that have escaped the disease; *B*, underground appearance of representatives of the two kinds. (Cuttings have been shortened for photographing.)

kinds of roots due to soil-fauna damage and root rot, and is slowly dying from moisture and nutrient deficiency. Many less severely damaged and surviving plants were subsequently attacked by *Marasmius stenophyllus*, as shown in figure 4. The whitish, matted mycelium has nearly completely enveloped the base of the shoots, cemented the leaf sheaths together, produced numerous superficial cortical cankers, and largely suppressed further root development.

While the association of *Marasmius* in the above sequence, as well as on ratoon cane, was common during several years' observations of the old varieties, it was obviously not a major factor in the disease complex. Often its appearance in fall-planted cane was limited to the small percentage of plants that had sprouted in the fall and suffered severe root rot during the subsequent winter and spring. Some badly root-rotted fields never showed it, while in others its extensive development was delayed until midsummer, with still less evident damage to the plants.

In stubble or ratoon fields, root rot often prevents or greatly delays sprouting of the stubbles, with resultant gappy stands and frequent failure. Although until late in the spring the stubble pieces may remain perfectly sound, deficiency of absorbing roots and low soil temperatures prevent a vigorous germination so characteristic of resistant varieties. Even when a satisfactory initial stand has been obtained, extensive death of plants may occur in April and May, due to root rot of the ratoon roots and red rot of the old stubbles on which the plants were still largely dependent.

The uncomplicated effect of root rot alone has been most striking in a number of hybrid seedlings not released for commercial culture that are very susceptible to this disease, but resistant to both red rot and mosaic. Some of these, giving excellent growth in the plant-cane crop, have completely failed to ratoon even in the best land; others have produced only occasional stalks too weak to sucker, which may be pulled up with but little exertion. The behavior of such varieties indicates that the conditions under which stubble crops become established are vastly more severe than is the case with plant cane. Typical instances of such seedlings are discussed later (pp. 75–76) under tests on seedling selections.

With the onset of high temperatures during June both plant and ratoon crops previously injured and retarded by root rot, with or without *Marasmius* accompaniment, put out new roots and, under favorable moisture and cultural conditions, make rapid growth. The activity of *Pythium arrhenomanes* is thereafter limited to cortex invasion and rootlet rot. Wherever the latter has attained serious proportions, the leaves show signs of wilting at midday during brief periods of drought even though the soil moisture is considerably above the wilting point for healthy plants. Examination of the roots reveals a general paucity of fine lateral rootlets, which have rotted and quickly disappeared under field conditions. However, the course of the disease is more easily followed in greenhouse-inoculation tests in steamed sandy garden soil. Plate 1, *A*, shows the typical rootlet rot and blackened cortex of main roots contrasted with healthy roots of the D–74 variety grown at a constant soil temperature of 30° C. (86° F.). Thus under midsummer temperatures, the *Pythium* is still able to damage seriously the root system of a very susceptible variety.

ON MODERATELY SUSCEPTIBLE AND RESISTANT VARIETIES

Except under extremely unfavorable conditions, *Pythium* root rot of moderately susceptible varieties, such as P. O. J. 36–M, P. O. J. 234, Co. 281, and C. P. 28/19, does not produce severe wilting, yellowing of the leaves, or death of young plants. The only aboveground symptoms are merely an unthrifty appearance with deficient and usually delayed suckering and closing-in over the middles or spaces between the rows. The ultimate result is found in reduced yields of millable cane and a shortening of the ratooning period or crop cycle. Unusually retarded ratoon, or stubble, fields are slower in ripening and sometimes, as explained above, yield less sugar per ton of cane than normally, later-maturing plant cane of more resistant varieties. Such is typical of their behavior on light lands and even on mixed and heavy lands following mild winters. Extensive loss of roots may occur, but the recuperative ability displayed by such varieties following the onset of hot weather is nothing less than amazing. Thus, fairly satisfactory yields are obtained.

The inherent susceptibility of these vigorous hybrid varieties is most strikingly revealed in plantings on mixed and heavy clay soils during a bad root rot year, which is characterized by prolonged periods of cold wet weather interspersed by warm spells during the months of February to June. Then they show the same symptoms and often as much damage as very susceptible varieties. Plates 1, *B*, and 2, *A* and *B*, illustrate the typical effect of root rot on representatives of this moderately susceptible group in heavy soils. Noting such a condition, the planters quickly recognize that these varieties are not adapted but are even hazardous for planting in such land.

With resistant varieties, such as Co. 290, C. P. 807, and C. P. 29/116, the condition illustrated in plate 2, *A* and *B*, has never been observed. However, death of younger plants and resulting gappy stands may follow destruction of the seed cane by red rot. All plants large enough to establish an independent root system prior to or at the time of the rotting of the mother cutting and death of its roots will appear healthy, although often backward in growth. Normally, whatever root rot that may occur on these varieties is largely limited to the finer laterals and often merely to their soft terminal portion. Sometimes a reddish zone separates the rotted from the sound whitish remainder of the rootlet, indicating a wound reaction and definite restriction of further invasion by the *Pythium*.

From the above account it is clear that the development, spread, and severity of root rot may be exceedingly variable and influenced by a multitude of factors, of which some favor the parasite and others the host. Sometimes these opposing factors are very evenly balanced throughout the season until near harvest when suddenly a fairly well-developed crop of a moderately susceptible variety may severely lodge and become uprooted, revealing for the first time the extent of destruction of its root system. In a few such cases investigated, which were readily distinguished from ordinary lodging, uniformly high moisture prevailing throughout the growing season had compensated for the reduction in root-absorbing area and had permitted development of a normal crop.

ETIOLOGY

In previous publications (*74*, *79*), *Pythium arrhenomanes* was shown to be the principal cause of root rot in Louisiana. However, a number of other species of *Pythium* and several other fungi have been isolated from rotted cane roots whose relation to the problem may now also be considered.

METHODS OF ISOLATION

Direct transfer to nutrient agar plates of thoroughly washed fragments of rotted roots, as first employed by the writers, was found to be unreliable for definite determination of the presence or absence of a suspected causal fungus. Quick development of molds and bacterial spreaders hindered or suppressed germination of *Pythium* oospores and rendered difficult the separation into pure culture of equal or slower growing organisms.

A modified technique suggested by Drechsler, whereby the period of washing is prolonged and the isolation pieces finally pressed dry between filter papers, gave less trouble with contaminants and has been extensively employed.

A still more certain, though indirect method, suggested by the junior author, consists in transferring each root rot collection with adhering soil, or even a few grams of the soil alone taken from the vicinity of rotted roots, to 5-inch pots of autoclaved garden soil, in which is planted a disinfected cutting of the very susceptible Louisiana Purple or other variety. After 2 to 3 weeks, under favorable greenhouse conditions, severe root rot usually develops, i. e., if *Pythium arrhenomanes* was present in either active or dormant condition in the original collection. Then, at the convenience of the investigator, the pots are washed out and freshly rotted root tips, after again washing in water, are transferred directly to corn-meal agar plates. Practically pure cultures of *Pythiums* or *Rhizoctonias* may then be subcultured from the rapidly developing thalli. In using this indirect method great precaution must, of course, be observed in the greenhouse to avoid contamination or spread of root rot organisms from one pot to another or from any outside source.

All cultures were then purified from bacterial or fungal contaminants (only in case the latter have a slower rate of growth) by transfers to the center of thick, plain agar plates. After a small thallus developed, the agar was inverted into the lid of the plate and new transfers made from the original bottom of the disks.

SURVEYS OF FUNGI ASSOCIATED WITH ROOT ROT

Two partial surveys of root-rotting organisms have been conducted in Louisiana as time from other activities permitted. The first, during the period 1927–31, involved examination of and collections from more than a hundred fields of the old varieties, D–74 and Louisiana Purple, that were failing from root rot and associated diseases, as well as collections from most of the increasing areas of P. O. J. 36, 213, and 234, which were rapidly supplanting the above canes. The second survey, made in 1935–36, was limited to about 20

representative fields, including many previously sampled, that were then in their first to third cropping cycle with one or another of the still more recently introduced varieties, Co. 281, C. P. 807, Co. 290, and C. P. 28/19.

Isolations during the first survey, involving several hundreds of root tissue platings, were made by the first and second above-described methods. A great variety of soil fungi, mostly well-known saprophytes, were obtained. However, many of the freshly rotted or flaccid root tips or rootlets yielded species of *Pythium*, *Rhizoctonia*, or *Fusarium* that were known to be pathogenic at least on other crops. Unfortunately, in 1929, a leaky ice box ruined many of these cultures before they had been determined. The remainder have been studied, and, with assistance from Charles Drechsler, all the *Pythiums* definitely identified. These are listed in table 1, to which is added some comparable measurements of rate of growth of mycelium and average diameter of oogonia in corn-meal agar with or without addition of "humic acid" previously described by the writers (*78*).

TABLE 1.—*Fungi isolated from rotted cane roots collected in representative fields throughout the Louisiana sugar district, together with some comparative measurements*

Name of fungus	Cultures	Radial growth in 48 hours at 30° C.	Diameter of oogonia [1]	
			Range	Mean
	Number	*Mm*	μ	μ
Pythium aphanidermatum (Edson) Fitzpatrick	2			
P. arrhenomanes Drechsler	201	59.8	21–35	27
P. complectens Braun	3			
P. complens Fischer	20	34.4	11–25	17
P. debaryanum Hesse	45	58.1	11–22	17
P. dissotocum Drechsler	57	47.4	17–28	22
P. graminicolum Subramaniam	20	48.2	19–28	23
P. irregulare Buisman	5			
P. mamillatum Meurs	18	75.8	11–16	15
P. periilum Drechsler	5			
P. ultimum Trow	13	80.7		
Rhizoctonia solani Kühn	110			
Fusarium (several species)	80			

[1] Measurements of 25 oogonia from a single representative culture of each species excepting *Pythium arrhenomanes* for which the figures are based on 42 Louisiana cultures reported in a previous publication (*79*).

In view of the circumstances described above, the number of cultures of the different species in table 1 does not, of course, reflect their relative abundance in fields examined nor even in the writers' original collection. They are merely the cultures on which the growth measurements were made and that were tested for pathogenicity. However, in the isolations of 1930, the species most frequently obtained, excepting *Pythium arrhenomanes*, were *P. dissotocum*, *P. debaryanum*, and *P. complens*. These and the remaining species, including *Rhizoctonia* and sometimes *P. arrhenomanes*, were noted to develop especially from water-soaked areas extending from the abundant lacerations or pits made in the soft terminal portions of the roots by snails, centipedes, springtails, and other minute soil fauna listed in a previous paper (*74*). Plate 1, *B*, illustrates typical material furnishing such cultures, as well as the combined damage to very young roots from a single shoot of P. O. J. 213 plant cane

collected April 16, 1928, near Houma, La. Isolations from decaying seed roots during the winter months also gave a variety of *Pythiums*, particularly damping-off types, such as *P. ultimum* and *P. mamillatum*, in this first survey.

Figure 5 presents outline tracings of the typical sex apparatus of several of the species from cane roots along with *Pythium arrhenomanes*, to show their principal identifying characteristics. *P. graminicolum* (fig. 5, *B*) is the only species here considered that bears any superficial resemblance to *P. arrhenomanes*. However, as previously pointed out by Drechsler (*33*), these two are readily separated by the usually larger numbers and more distant origin of the antheridia of the latter, which may be classified as diclinous, whereas the former is more typically monoclinous. These characters are easily determined by a study of thick microscopic sections of fruiting cultures in 2-percent agar medium.

Asexual characters are usually highly variable, although after some experience are valuable adjuncts in identification of cultures. However, in dissected root tissue the lobulate sporangia of *Pythium complens* and *P. graminicolum* have been observed and found more or less similar to those produced by *P. arrhenomanes*.

While the results of preliminary inoculation tests indicated that *Pythium arrhenomanes* was the only species displaying a high degree of virulence toward cane roots, only about 50 of the *Pythium* cultures from the writers' first survey could be identified as this species. The ease with which it was obtained from some collections, particularly those showing very fresh root rot following a rain, suggests that the low incidence of success in other attempts was due more to the condition and age of the material and inadequate methods than to actual scarcity of *P. arrhenomanes*. This conclusion is supported by the results of the 1935 and 1936 surveys, in which the third above-described method of isolation was employed. Out of 76 root rot collections of these later surveys that were sent to Washington and added to pots of steamed soil growing a susceptible variety, 42, or 55 percent, resulted in severe root rot from which *P. arrhenomanes* and no other *Pythium* was isolated. The percentage would doubtless have been higher had not more than two-thirds of the collections been made from the highly resistant varieties Co. 290 and C. P. 807. While these varieties rarely show appreciable root rot, the fungus had readily survived, as proved at least for two large fields where C. P. 807 had been grown for 7 years, or through two crop cycles.

Approximately 150 of the cultures listed in table 1 for *P. arrhenomanes* were obtained from this second survey. Forty-two of the remainder, from the first survey, were studied in detail with respect to variability and physiologic specialization, reported in an earlier paper (*79*). Additional cultures of *P. arrhenomanes* were also isolated from sugarcane in several Everglades muck-soil areas of Florida where rootlet rot is common, but of no importance, save possibly in raw sawgrass land which has not received the copper-manganese treatment required for normal cane growth. It was exclusively from these areas that occasional cultures of *P. butleri* and *P. helicoides* Drechsler were isolated.

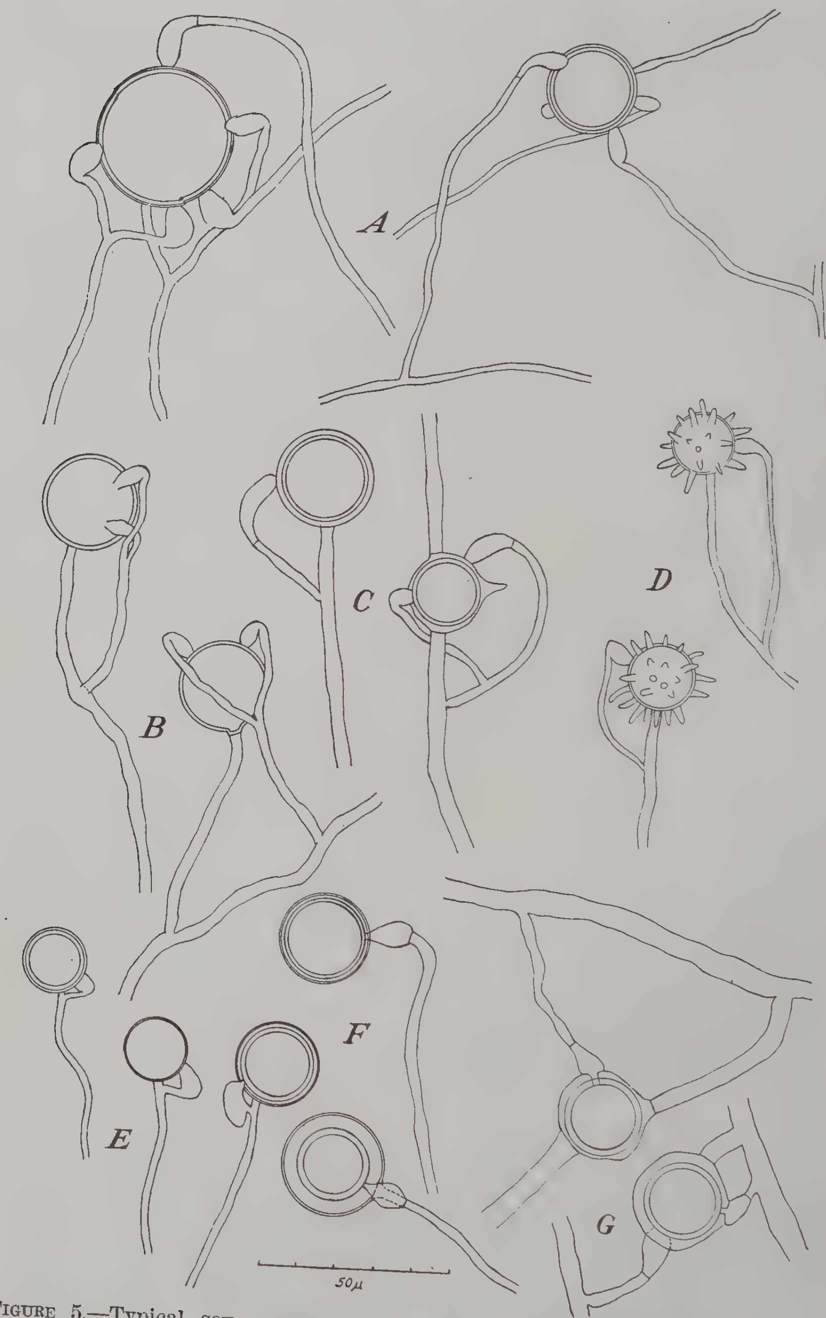


FIGURE 5.—Typical sex apparatus of six species of *Pythium* isolated from sugarcane roots, showing diagnostic characters distinguishing them from *Pythium arrhenomanes*: *A*, Two strains of *P. arrhenomanes*, illustrating extremes in size of oogonia; *B*, *P. graminicolum*; *C*, *P. debaryanum*; *D*, *P. mamillatum*; *E*, *P. complens*; *F*, *P. ultimum*; and *G*, *P. dissotocum*. (Outline tracings by aid of camera lucida.)

Edgerton and Tims (*38*), Tims and Mills (*92*), and Edgerton, Tims, and Mills (*40*) have reported the results of several systematic surveys of fungi on cane roots in Louisiana. Root rot collections were obtained in 1927 from 263 fields in all parts of the sugarcane belt. Of the 7,890 roots cultured, forms of *Pythium* were observed in 1,480, which indicate the wide distribution and abundance of members of this genus in Louisiana soils. A smaller survey in January and February 1928 yielded 967 cultures of *Pythium*, of which approximately 80 percent was found to be parasitic, to some extent at least, on corn roots by both the Petri-dish and pot methods of testing. While their cultures were not definitely identified, these authors emphasized that the most virulent ones (proportion not given) on both cane and corn showed many similarities to the cane fungus described by Carpenter (*24*), in Hawaii, and since assigned to *P. arrhenomanes*. The surveys by these workers and by the writers leave no doubt that *P. arrhenomanes* is the principal species associated with root rot of sugarcane in Louisiana.

One other species with spiny oospores is sufficiently described by both Carpenter and Edgerton and associates to suggest its identity with occasional cultures also obtained by the writers. These were at first (*74*) identified as *Pythium spinosum* Sawada, but more recently, after comparison of cultures, Drechsler has assigned them to *P. mamillatum*. Occasional cultures of an apparently undescribed species obtained in 1927 and named *P. dactyliferum* by Drechsler have since been identified by him as *P. irregulare* Buisman. *P. dissotocum*, *P. periilum*, and the water mold *Plectospira gemmifera* (not listed in table 1) are all new species described by Drechsler (*31*, *32*) from the writers' sugarcane collection.

PATHOGENICITY OF MISCELLANEOUS FUNGI COMPARED WITH PYTHIUM ARRHENOMANES

The frequency (table 1) with which several of the *Pythiums*, *Rhizoctonias*, and *Fusaria* were isolated in comparison with *P. arrhenomanes*, in the writers' first survey, suggested that the latter fungus might not be solely responsible for sugarcane root rot. Preliminary inoculation tests with these miscellaneous fungi have been negative so far as the development of a general root rot characteristic of *P. arrhenomanes* is concerned. However, they have been readily reisolated and reidentified from occasional rotted root tips and rootlets. Therefore, in a previous publication (*74*) they were listed as "wound infecting or weakly parasitic forms." This tentative conclusion was based on their apparent association with, or dependence upon, soil-fauna damage to the roots or to an unhealthy condition, in case of seed roots, produced by red rot invasion of the cutting.

A few of the greenhouse experiments to determine their relative pathogenicity under a wide range of environmental conditions may be summarized.

COMPARISON OF EIGHT SPECIES WITH PYTHIUM ARRHENOMANES (EXPERIMENT 1)

An experiment was carried on from May 24 to June 27, 1927, to compare *Pythium arrhenomanes* with eight other species of root rot

fungi. Twenty 6-gallon garbage cans were filled with a rich garden soil that had been pasteurized in trays by subjection to 5 pounds' steam pressure for 1 hour. Four two-joint cuttings of the very susceptible Louisiana Purple variety, grown in the greenhouse, were surface-disinfected and planted in each can. Two cans were then inoculated with Petri-dish cultures of separate collections of each of the following fungi: *Pythium arrhenomanes*, *P. complectens*, *P. complens*, *P. dissotocum*, *P. graminicolum*, *P. mamillatum*, *P. periilum*, *Plectospira gemmifera*, and *Rhizoctonia solani*. Two cans were left uninoculated for controls. Additional controls, consisting of one steamed and one unsteamed can, contained soil recently shipped from a Louisiana plantation field that had not grown sugarcane for 10 years. Soil temperatures during the test ranged between 14° and 29.5° C., with an average of 22°.

After 18 days, one to five plants had come up in all the cans, those inoculated with *Pythium arrhenomanes* being fewer and more backward than any of the others, except the unsteamed control containing Louisiana soil that appeared to be suffering from nitrogen deficiency. After approximately 1 month, the soil of all cans was washed out and the root systems were examined. Those that had been exposed to *P. arrhenomanes* showed very severe damage and about one-fourth the mass of the checks; most of the seed roots had been destroyed on emergence, and others were largely devoid of rootlets; black specks on shoot roots indicated where rootlets had emerged and rotted. Destruction of seed roots had apparently permitted fermentation and decay of the cuttings.

Plants exposed to the remaining fungi showed excellent, nearly complete whorls of seed roots with many fine laterals, but as yet very few shoot roots, due apparently to adequate functioning of the temporary root system. The middle internode of the seed cuttings was still sound in most cases. Closer examination of the roots revealed extensive root-tip rotting of these plants exposed to *Pythium dissotocum* and *P. mamillatum;* those with the *Rhizoctonia* showed, in addition to decayed root tips, brownish-black lesions and some distortion of the larger roots of the type previously described by Matz (*66*), Bourne (*16*), and Edgerton, Taggart, and Tims (*37*). The root systems that had been exposed to *P. complectens*, *P. complens*, *P. graminicolum*, *P. periilum*, and *Plectospira gemmifera* showed only an occasional flaccid root tip. This could not be considered significant because careful search revealed also a few in the controls, including the unsteamed plantation soil.

Reisolations of fungi from representative rotted roots were attempted for all cans. The fungi were successfully reisolated in pure culture and identified in all cases except *Pythium complectens* and the water mold *Plectospira gemmifera*. A pinkish *Fusarium* and common molds, apparently from aerial contamination of the cans, developed from many of the tissue plantings and exclusively in the steamed controls. From the unsteamed and uninoculated Louisiana soil, *Pythium complens* was the only *Pythium* obtained.

Under the conditions of this qualitative, or observational, test *Pythium arrhenomanes* was the only organism displaying pronounced parasitic tendencies.

COMPARATIVE VIRULENCE OF PYTHIUM APHANIDERMATUM, P. DISSOTOCUM, P. GRAMINICOLUM, AND P. ARRHENOMANES (EXPERIMENT 9)

An experiment was conducted from June 6 to September 27, 1928, to compare *Pythium aphanidermatum*, *P. dissotocum*, and *P. graminicolum* with *P. arrhenomanes* on the tolerant variety P. O. J. 36–M. Steamed garden soil was placed in 12-inch flowerpots, and in each pot a single three-joint cutting was planted. Five pots were then inoculated with each fungus and five left uninoculated as controls.

After approximately 2 months, one pot of each series was washed out. The control had excellent whorls of healthy, white seed roots and well-developed shoot roots; the *Pythium dissotocum* and *P. graminicolum* pots were alike with many flaccid root tips and some general browning of rootlets, while *P. aphanidermatum* showed much less evidence of infection. Viewed at a distance of 10 feet, the root systems of all three inoculated plants were indistinguishable from the control and formed a striking contrast with the plant exposed to *P. arrhenomanes.* The seed roots of the latter were mostly brown and dead, and the terminal portions of the fine rootlets arising from the shoot roots were rotted.

Root examinations on conclusion of the experiment (after a growth period of 4 months) revealed noticeable effect of inoculation only by those plants exposed to *Pythium arrhenomanes.* Even with the latter there was no general invasion, although numerous reddish lesions occurred on the larger roots that, under the prevailing high summer temperatures, had not been greatly damaged nor retarded in growth. Therefore, in comparison with experiment 1, the more resistant variety and the higher temperatures of this test had separately, or jointly, retarded infection of rootlets and restricted the spread of cortex lesions. The four-pot average fresh weight of tops were for controls, 605 g; *P. aphanidermatum*, 634 g; *P. dissotocum*, 761 g; *P. graminicolum*, 792 g; and *P. arrhenomanes*, 510 g. Thus, the last-mentioned alone caused a barely significant reduction (15.7 percent) below controls. This was mainly due to fewer and smaller suckers on the diseased plants.

COMPARISON OF EIGHT ISOLATES OF PYTHIUM GRAMINICOLUM (EXPERIMENT 11)

In an experiment conducted from October 10, 1928, to February 6, 1929, eight cultures of *Pythium graminicolum*, from as many different Louisiana canefields, were compared on the Louisiana Purple variety of sugarcane and on Yellow Creole corn. Materials and procedures were the same as in experiment 9. Examinations and weights after 3 months revealed no significant differences between the isolates in yield nor damage to the roots of either crop, but in all cases there could be readily found some rootlet tip rotting and grayish to brownish lesions on larger roots, particularly of the corn plants, which, on reisolation, yielded practically pure cultures characteristic of *P. graminicolum.*

TESTS OF THREE SPECIES AS POSSIBLE SECONDARY INVADERS (EXPERIMENT 24)

In order to determine whether the more numerous species of *Pythium*, other than *P. arrhenomanes*, listed in table 1 might have been secondary invaders to the latter fungus, a series of separate and

combination inoculations with the latter fungus were conducted from March 3 to May 13, 1931. Ten cans of steamed garden soil were prepared as in experiment 1, and each was planted with two three-joint cuttings of Louisiana Purple and Co. 281. Inoculations were made at the time of planting and were from separate pure cultures of the various species grown on corn-meal-sand medium. Mildly parasitic and virulent strains, Nos. 11 and 58, respectively, of *P. arrhenomanes* were used singly in cans 1 and 2, and each were used in the following combinations: in cans 3 and 4 *P. dissotocum*, in cans 5 and 6 *P. complens*, and in cans 7 and 8 *P. debaryanum;* in can 9 all three of the last mentioned were used together, and can 10 was left uninoculated as a control.

After approximately 1 month, during which soil temperatures ranged between 20° and 28° C., reisolations were commenced and were continued over another month. Comparisons of root systems revealed severe rotting of both varieties in all cans in which *Pythium arrhenomanes* culture 58 had been added; it was less severe in those receiving culture 11, and here the greater resistance of Co. 281 was more noticeable. Can 9, in which neither of these cultures was used, showed only a very small amount of root rot on both seed and shoot roots typical of earlier tests with these three species of *Pythium*. The control roots were white and healthy. From all inoculated cans a total of 504 roots were plated out and 493 cultures of fungi were obtained, of which 227 were *Pythiums*. The remainder consisted of various molds and *Fusaria*. Of the total *Pythiums*, 220 were determined as *P. arrhenomanes*. No consistent difference was found between the two varieties in kind and number of *Pythiums* isolated from freshly rotted root tips. Wherever *P. arrhenomanes* had been added, it was, with two exceptions, the only species of *Pythium* reisolated. From can 7 receiving *P. debaryanum* and culture 58 of *P. arrhenomanes*, 1 culture of the former and 36 of the latter species were reisolated. One culture of *P. dissotocum* and 15 cultures of *P. arrhenomanes* (culture 11) were reisolated from the cans receiving this combination. Another culture of *P. dissotocum* and four of *P. complens* were obtained from 50 scattered infections in can 9, in which *P. arrhenomanes* was omitted. No mixture of species was noted on any of the reisolation cultures. That this might be the result of competition and domination of one over the other seemed unlikely, since at least three of the species showed similar growth rates in culture (table 1). Thus, none of these mildly parasitic species of *Pythium* was revealed in the role of secondary invader to *P. arrhenomanes*. Apparently wherever the latter fungus was present it was, in practically all cases, the sole cause of root rot.

RHIZOCTONIA AND MARASMIUS AS POSSIBLE SECONDARY INVADERS (EXPERIMENT 68)

Possible secondary invasion by *Rhizoctonia* and *Marasmius*, following *Pythium arrhenomanes*, was tested in an experiment carried on from February 24 to May 27, 1936, by the same procedure as used in experiment 24. However, Louisiana Purple alone was employed as the host and previously sprouted, uniform plants were reset in 3-gallon crocks of steamed garden soil with each object tested in duplicate to obtain preliminary yield comparisons. Two strains of

P. arrhenomanes of differing virulence were compared individually and in the combinations listed in table 2, which gives also the green weight of tops and percentage differences from the uninoculated controls.

TABLE 2.—*Separate and combination inoculations of the Louisiana Purple variety with Pythium arrhenomanes and other fungi isolated from sugarcane roots*

Species used	Mean weight	Decrease (−) or increase (+) from inoculation
	Grams	*Percent*
None (control)	625	
Pythium arrhenomanes (No. 58) alone	420	−33
Pythium arrhenomanes (No. 58) plus *Marasmius stenophyllus*	380	−39
Marasmius stenophyllus alone	594	−5
Pythium arrhenomanes (No. 1557) alone	197	−68
Pythium arrhenomanes (No. 1557) plus *Rhizoctonia solani* (No. 1570)	208	−67
Rhizoctonia solani (No. 1570) alone	716	+15
Rhizoctonia solani (No. 945) alone	684	+9
Rhizoctonia sp. (No. 1572 small sclerotia) alone	678	+8

Important reductions in yield were obtained only where *Pythium arrhenomanes* was included; the addition of the other fungi had no significant effect. The *Rhizoctonias* had caused occasional brownish lesions on the larger roots and *Marasmius* an infrequent superficial cortical lesion on the rhizome. Mycelial mats of the latter fungus had formed beneath the older dying leaf bases, especially on the duplicate set of plants, which had been hilled up about 4 inches a month before harvest. Therefore, no evidence was obtained that either of the latter fungi takes particular advantage of a weakened condition of the plant produced by *P. arrhenomanes.*

SOIL TOXINS ACCENTUATING DAMAGE BY MILDLY PARASITIC SPECIES

EXPERIMENT 34

In a previous article (*80*) the question of possible harmful soil constituents, or toxins, accumulating in poorly drained or continuously cropped cane lands and their influence on root susceptibility is discussed. In view of the increased root damage by *Pythium arrhenomanes* resulting particularly from small additions of salicylic aldehyde to the nutrient solution, it seemed desirable to determine whether the predisposing effect of this substance would facilitate root infection by the otherwise very mildly parasitic species of *Pythium* tested above. Six-week-old uniform plants of the Louisiana Purple variety, sprouted in 3-inch pots of steamed quartz sand, were selected and transplanted on April 27, 1932, to a series of 3-gallon stone crocks also filled with autoclaved quartz sand. Seven-day-old cultures of the fungi and an equal quantity of sterile medium in the controls were mixed in the top 4 inches during resetting. The crocks were then watered with 4 liters of a standard nutrient solution and handled according to the method previously described by the writers (*79, 80*). Salicylic aldehyde, at the rate of 50 parts per million of liquid in the receiving jar only, was added twice weekly to all crocks from May 2 to June 6, during which period the nutrient solution was completely renewed four times.

Table 3 shows the fungi tested, total elongation of primary shoots, and the green weight of tops when harvested on June 6, 1932. Severe reduction in plant weight was caused by *Pythium graminicolum* and significant reduction by *P. dissotocum*, which are in marked contrast with the mild behavior of these species in the absence of toxin additions in previous experiments. The root systems exposed to *P. graminicolum* were greatly reduced and discolored by root-tip rot and extensive pinkish cortex lesions; those exposed to *P. dissotocum* showed also much root rot, and this was progressively less with *P. complens*, *P. ultimum*, and *P. debaryanum*. The root mass of those plants exposed to *P. debaryanum* was fully as large as the controls, as shown by representative specimens in figure 6. Root systems of the controls appeared normal and healthy, with an abundance of white roots. The respective fungi were reisolated from each inoculated series except *P. ultimum* and *P. dissotocum*, the isolation plates of which were overrun by *Trichoderma*. In general, the species causing greatest damage in this test in the presence of salicylic aldehyde were the ones producing most numerous infections, but insignificant damage, in the earlier tests without the addition of aldehyde.

Table 3.—*Relative damage by otherwise mildly parasitic species of Pythium to the Louisiana Purple variety grown in a nutrient solution receiving periodic additions of 50 p. p. m. of salicylic aldehyde*

Species used	Mean height of plants (centimeters)	Mean green weight of tops [1] (grams)	Reduction due to inoculation [1]	
			Grams	Percent
None (control)	16.1	99.0		
Pythium complens	13.2	88.1	10.9	11.0
P. debaryanum	15.0	103.5	[2]+4.5	[2]+4.5
P. dissotocum	12.8	71.3	27.7	27.9
P. graminicolum	11.4	58.2	40.8	41.3
P. ultimum	15.5	82.3	16.7	16.8

[1] Differences between means (of 4 replications) required for odds of 19:1=18.6 g, or 18.6 percent of controls.
[2] Increase.

EXPERIMENT 56

The surprisingly large amount of root rot caused by the "mildly parasitic" *Pythiums* in the presence of salicylic aldehyde in the preceding experiment made further studies desirable. Limited greenhouse space and quantity of distilled water available for that test prevented inclusion of a series of *Pythium arrhenomanes* as well as untreated controls. Furthermore, the initially high, and subsequently fluctuating, concentrations of the toxin were unsatisfactory. Therefore, a further test, employing new sand, was set up on February 14 and continued to May 7, 1935, to which all of the 4 liters of nutrient (the capacity of crock and drain jar) received, in the treated series, 10 p. p. m. of salicylic aldehyde.

In this experiment it was sought to determine the effect of a low concentration of the aldehyde on infection and damage by *Pythium dissotocum* (isolate No. 36–2) in comparison with *P. arrhenomanes* (isolate No. 58) on the moderately susceptible variety, Co. 281. A uniform lot of plants about 10 inches tall growing in 4-inch pots of

FIGURE 6.—Relative damage by mildly parasitic species of *Pythium* to the Louisiana Purple variety grown in sand culture in the presence of salicylic aldehyde: *A*, Control, showing development of suckers; *B*, inoculated with *P. debaryanum; C*, with *P. complens; D*, with *P. dissotocum; E*, with *P. graminicolum.*

steamed sand was selected and reset, one plant per crock. Each crock was then leached with 4 liters of distilled water followed by the addition of 2 liters of nutrient solution. After 3 days, inoculations were made by placing one-half teaspoon of sand-corn-meal culture in each of two holes 3 inches deep near to, and on the opposite side of, each plant, and a similar quantity of sterile medium was used in the controls. The six crocks of each of the six treatments were distributed in Latin square arrangement on the greenhouse racks. After another 3 days, 4 liters of nutrient solution, with 10 p. p. m. of salicylic aldehyde as required, were added to each crock and jar. Daily thereafter the contents of the jars were poured over the crocks, as in the preceding experiment. Every 2 weeks the old solution was completely replaced with new nutrient and toxin. This interval, based on calculations from published plant analyses, provided an ample supply of nutrient salts for normal and rapid cane growth. The reaction of leachings varied between pH 4 and 6. Thrice daily temperature readings 3 inches below the surface of the sand varied between 16° and 35° C., with an average of 23.5° for the entire period of the experiment.

The results are summarized in table 4, which shows that a low concentration of salicylic aldehyde alone had no appreciable effect on the severity of root rot caused by *Pythium arrhenomanes*. The combination with *P. dissotocum* gave an 11-percent reduction in green weight, which, however, is not statistically significant because of the rather high variability of the test as a whole. Examination of root systems showed that considerable damage by both fungi had occurred during the early part of the test when sand temperatures were at 20° C. or below. Therefore, from this and preceding experiments it may be inferred that the presence in soils of subtoxic concentrations of salicylic aldehyde, or other substances having a similar effect, may indirectly promote the multiplication of otherwise mildly parasitic species of *Pythium* and bring damage to plants of moderately susceptible varieties.

TABLE 4.—*Effect of 10 p. p. m. of salicylic aldehyde (toxin) on root infection and damage by Pythium dissotocum in comparison with P. arrhenomanes on the "tolerant" sugarcane variety, Co. 281*

Treatments and fungi compared	Mean total height of plants	Mean green weight of tops [1]	Reduction from control (series 1)[1]
	Centimeters	*Grams*	*Percent*
None (control)	106.2	387.8	
Toxin alone	102.3	394.5	[2]+1.7
Pythium arrhenomanes alone	84.4	295.8	23.7
P. arrhenomanes plus toxin	91.8	298.0	23.2
P. dissotocum alone	102.7	385.8	.5
P. dissotocum plus toxin	96.3	344.3	11.2

[1] Differences between means required for odds of 19:1 equal 103 g, or 26.5 percent of controls.
[2] Increase.

While the above-tested and probably still other miscellaneous species of *Pythium* and other soil-inhabiting fungi may under certain abnormal conditions destroy sugarcane roots, results of these

inoculation tests, in conjunction with the survey records, suggest that these organisms are of minor importance compared with *Pythium arrhenomanes*.

PHYSIOLOGIC SPECIALIZATION OF PYTHIUM ARRHENOMANES

VARIABILITY AMONG INDIVIDUAL ISOLATES

A detailed study of both physiologic and morphologic variability in *Pythium arrhenomanes* was reported in an earlier paper (*79*). Data were given on a collection of 70 isolates mainly from sugarcane in Louisiana, but including representatives from the Corn Belt, cereal root rot in Canada, and sugarcane root rot in Hawaii and Mauritius, all of which were compared in culture, and the local forms in pathogenicity.

The results of the study showed that although any given isolate maintained fairly constantly its individual peculiarities in both cultural characters and degree of virulence, wide differences between isolates, even from the same locality, were found to be characteristic of the species. Extreme differences in virulence on the same or different varieties and lesser differences in reaction to temperature and changes in pH value indicated the potential adaptability of the fungus to changes in both host and environment.

DIFFERENCES IN VIRULENCE BETWEEN LOCALITIES AND SURVEYS

It has been the purpose of further studies to determine whether important differences in virulence of the fungus exist between different localities and individual plantations or fields within the relatively small area of the Louisiana sugar district. Any significant locality differences might explain some of the variation in damage from root rot in commercial fields, as well as have an important bearing on tests of seedlings for root-rot resistance, which are at present confined largely to experiment-station farms. A further important object was to detect and measure any general shift in virulence of the fungus that may have occurred in recent years in response to the continual introduction of new varieties varying widely in genetic constitution, but usually somewhat more resistant to root rot than those displaced.

COMPARISONS ON INBRED CORN

The comparison of large numbers of cultures in the limited greenhouse space necessitated dependence mainly upon corn rather than sugarcane as the test host. For this purpose the suitability of a highly susceptible selfed line (No. 608–325) of the Lancaster Surecrop variety was shown in a previous publication (*79*). This particular selection, originally supplied as the F_8 inbred generation by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, has been grown each summer under isolation and selfed without further selection to furnish a continually viable supply of seed. F_{10} seed was planted in experiment 63 and the F_{12} in experiment 67. It has shown great uniformity in physical characters and susceptibility

to root rot. Selfed lines of other varieties of corn have been used in preliminary tests, but showed no particular advantage over the selection employed.

The detailed procedure followed in these comparisons was practically the same as reported in the writers' earlier inoculation tests (*79, p. 204*). It consists essentially in growing the plants for a period of 5 to 8 weeks after inoculation in 5-inch pots of quartz sand watered with a nutrient solution. The results are more or less reproducible in successive tests with the same host and may be compared over a period of time for consistency of behavior of the various *Pythium* cultures.

FIGURE 7.—General view of experiment 67 comparing relative virulence of 136 isolates of *Pythium arrhenomanes* on an F_{12} inbred selection of the Lancaster Surecrop variety of corn growing in sand-nutrient cultures. Photographed 1 week before harvest.

EXPERIMENTS 63 AND 67

In experiment 63, 44 cultures of the 1927–31, or first, survey from widely scattered localities were compared with 47 cultures of the 1935–36, or second, survey obtained mainly from Lafourche and Terrebonne Parishes, where root rot was being intensively studied. In experiment 67 the same 44 cultures of the first survey were compared with 92 additional cultures of the second survey that were selected at random from the collections from six parishes, including Terrebonne and Lafourche, making a total of 139 cultures of the second survey tested on corn.

In experiment 63, 10 pots were inoculated with each of the 91 cultures, and 10 control series of 10 uninoculated pots each were randomized among them in 10 experimental blocks on slat benches in the

greenhouse; experiment 67 had 7 replications of 136 isolates and 9 uninoculated series arranged similarly. The former extended during the sunshiny fall period, September 17 to November 8, 1935, which favored the growth of controls and also resulted in nearly double the percentage reduction by root rot than obtained during an equal but mostly cloudy period of 50 days in the latter test, extending from February 4 to March 26, 1936. Figure 7 is a general view of experiment 67 taken 1 week prior to harvest. Despite the proximity of the pots containing different inoculum, care in application of nutrient and water apparently prevented spread of the fungus from pot to pot, as shown by the invariably healthy root systems of the numerous controls interspersed among them. The mean green weights of tops and percentage reductions by root rot are given in table 5 and will be discussed later (pp. 36–42) in conjunction with similar tests on sugarcane.

TABLE 5.—*Greenhouse inoculation experiments on inbred corn and sugarcane comparing 215 cultures of Pythium arrhenomanes from various localities in the Louisiana sugar district*

Parish and culture No.	Year isolated	Source of culture			Lancaster Surecrop corn				Louisiana Purple sugarcane			
					Experiment 63		Experiment 67		Experiment 71		Experiment 84	
		Farm or locality	Variety	Crop	Mean weight	Reduction from controls	Mean weight	Reduction from controls	Mean weight	Reduction from controls	Mean weight	Reduction from controls
					Grams	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
Controls					23.5		18.6		669.6		666.2	
Assumption:												
379	1928	Glenwood	P. O. J. 234	Plant cane	19.1	18.7	13.8	25.8			227.1	65.9
380	1928	do	do	do	10.8	54.0	13.4	28.0			286.7	57.0
382	1928	do	do	do	8.9	62.1	12.1	34.9			227.2	65.9
Mean					12.9	45.1	13.1	29.6			247.0	62.9
1650	1936	Glenwood	Co. 281	First ratoon							157.2	76.4
1652	1936	do	do	do							215.9	67.6
1655	1936	do	do	do							196.4	70.5
Mean											189.8	71.5
Avoyelles:												
1605	1936	Bunkie	P. O. J. 213	Second ratoon							277.6	58.3
1606	1936	do	do	do							266.6	60.0
1607	1936	do	do	do							314.0	52.9
1608	1936	do	do	do							200.6	69.9
1609	1936	do	do	do							201.1	69.4
1611	1936	do	do	do							201.4	69.8
1613	1936	do	do	do							242.6	63.6
1614	1936	do	Co. 281	do							243.3	63.5
1615	1936	do	do	do							204.3	69.3
1616	1936	do	do	do							324.8	51.2
Mean											247.9	62.8

Lafayette:												
1479	1935	Billeaud	Co. 290	First ratoon			12.6	32.3				
1480	1935	do	do	do			12.6	32.3				
1481	1935	do	do	do			15.2	18.3				
1482	1935	do	do	do			13.1	29.6				
1487	1935	do	do	Plant cane			15.6	16.1				
1488	1935	do	do	do			15.0	19.4				
1489	1935	do	do	do			16.7	10.2				
1490	1935	do	do	do			12.0	35.5				
1504	1935	do	P. O. J. 36	First ratoon			13.4	28.0				
1506	1935	do	do	do			12.1	34.9				
1507	1935	do	do	do			12.4	33.3				
1508	1935	do	do	do			13.6	26.9				
Mean							13.7	26.3				
1501	1935	Landry	C. P. 807	First ratoon			17.1	8.1				
1502	1935	do	do	do			15.2	18.3				
1503	1935	do	do	do			17.2	7.5				
Mean							16.5	11.3				
Lafourche:												
348	1928	Greenwood	Louisiana Purple	Plant cane	16.5	29.8	15.6	16.1	305.0	54.5	325.8	51.1
350	1928	do	do	do	25.2	+7.2	18.5	.5	496.0	25.9	525.9	21.1
352	1928	do	do	do	14.6	37.9	11.5	38.2	319.7	52.3	357.0	46.4
354	1928	do	do	do	16.5	29.8	16.1	13.4	291.9	56.4	460.7	30.8
356	1928	do	do	do	20.3	13.6	17.9	3.8	517.0	22.8	429.1	35.6
Mean					18.6	20.9	15.9	14.5	385.9	42.4	419.7	37.0
1429	1935	Greenwood	C. P. 807	Plant cane	12.9	45.1			103.9	84.5		
1430	1935	do	do	do	9.8	58.3						
1431	1935	do	do	do	9.9	57.9			209.6	68.7		
1432	1935	do	do	do	10.1	57.0			198.9	70.3		
1433	1935	do	do	do			10.4	44.1	136.1	79.7		
1434	1935	do	do	do	9.0	61.7			198.6	70.3		
1574	1936	do	C. P. 28/19	do							267.4	59.9
1576	1936	do	do	do							257.6	61.3
1578	1936	do	do	do							349.4	47.6
1580	1936	do	do	do							194.9	70.7
1581	1936	do	do	do							212.9	68.0
Mean					10.3	56.2	10.4	44.1	169.4	74.7	256.4	61.5
1543	1935	Georgia	P. O. J. 36–M	Plant cane			12.2	34.4				
1544	1935	do	do	do			9.8	47.3	140.6	79.0		
1546	1935	do	do	do			14.5	22.0	169.6	74.7		
1547	1935	do	do	do			13.2	29.0				

TABLE 5.—*Greenhouse inoculation experiments on inbred corn and sugarcane comparing 215 cultures of Pythium arrhenomanes from various localities in the Louisiana sugar district*—Continued

Parish and culture No.	Year isolated	Source of culture			Lancaster Surecrop corn				Louisiana Purple sugarcane			
					Experiment 63		Experiment 67		Experiment 71		Experiment 84	
		Farm or locality	Variety	Crop	Mean weight	Reduction from controls	Mean weight	Reduction from controls	Mean weight	Reduction from controls	Mean weight	Reduction from controls
					Grams	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
Lafourche—Continued.												
1553	1935	Georgia	P. O. J. 36-M	Plant cane			10.9	41.4				
1555	1935	do	do	do			14.1	24.2				
1556	1935	do	do	do			13.3	28.5	143.5	78.6		
1557	1935	do	do	do			13.1	29.6				
1558	1935	do	do	do			11.5	38.2	155.8	76.7		
1559	1935	do	do	do			13.0	30.1				
1560	1935	do	do	do					112.7	83.2		
1563	1935	do	do	do					83.0	87.6		
Mean							12.6	32.3	134.2	80.0		
1421	1935	Raceland	C. P. 28/19	Plant cane	11.7	50.2						
1422	1935	do	do	do			9.3	50.0				
1423	1935	do	do	do	10.3	56.2						
1424	1935	do	do	do	10.8	54.0						
1425	1935	do	do	do			13.8	25.8				
1426	1935	do	do	do	10.7	54.5						
1427	1935	do	do	do	10.7	54.5						
1428	1935	do	do	do			10.4	44.1				
1435	1935	do	do	do			10.4	44.1				
1436	1935	do	do	do	7.4	68.5						
1445	1935	do	C. P. 807	do	10.0	57.4						
1446	1935	do	do	do			10.8	41.9				
1447	1935	do	do	do			10.7	42.5				
1448	1935	do	do	do	12.1	48.5						
1449	1935	do	do	do	7.8	66.8						
1450	1935	do	do	do	9.0	61.7						
1419	1935	Upper Ten	Co. 281	do	13.8	41.3						
1420	1935	do	do	do	10.3	56.2						
Mean					10.4	55.7	10.9	41.4				

Iberia:												
1598	1936	Albania	Co. 281	First ratoon							232.4	65.1
1599	1936	do	do	do							177.2	73.4
1600	1936	do	do	do							187.2	71.9
1601	1936	do	do	do							162.6	75.6
1602	1936	do	do	do							264.8	60.3
1603	1936	do	do	do							240.3	63.9
Mean											210.8	68.4
Pointe Coupee:												
1278	1931	Alma	Co. 281	Plant cane	9.3	60.4	12.2	34.4	112.3	83.2		
1279	1931	do	do	do	11.1	52.8	11.8	36.6	171.8	74.3		
1281	1931	do	do	do	14.3	39.1	16.3	12.4	275.0	58.9		
1284	1931	do	P. O. J. 234	do	10.9	53.6	12.8	31.2	161.2	75.9		
1285	1931	do	do	do	10.1	57.0	13.1	29.6	212.2	68.3	272.5	59.1
1295	1931	do	do	do	8.6	63.4	8.5	54.3	88.2	86.8	301.2	54.8
1296	1931	do	do	do	11.2	52.3	15.0	19.4	293.3	56.2	284.0	57.4
1320	1931	do	C. P. 807	do	10.6	54.9	12.3	33.9				
1323	1931	do	do	do	11.9	49.4	14.3	23.1				
1324	1931	do	do	do	11.4	51.5	11.1	40.3				
Mean					10.9	53.6	12.7	31.7	187.7	72.0	285.9	57.1
1509	1935	Alma	C. P. 28/19	Plant cane			16.5	11.3				
1510	1935	do	do	do			16.3	12.4				
1511	1935	do	do	do			18.1	2.7				
1522	1935	do	Co. 281	First ratoon			17.1	8.1	176.0	73.7		
1523	1935	do	do	do			12.5	32.8	132.3	80.2		
1524	1935	do	do	do			14.2	23.7	160.5	76.0		
1525	1935	do	do	do			15.5	16.7	171.6	74.4		
1530	1935	do	C. P. 28/19	do			13.0	30.1	216.5	67.7		
1531	1935	do	do	do			12.8	31.2	150.2	77.6		
1533	1935	do	do	do			14.3	23.1				
1534	1935	do	Co. 290	Plant cane			12.7	31.7			361.7	45.7
1535	1935	do	do	do			15.4	17.2			251.1	62.3
1537	1935	do	C. P. 29/320	do			13.0	30.1			255.7	61.6
1538	1935	do	do	do			14.5	22.0				
1539	1935	do	do	do			17.6	5.4				
1540	1935	do	do	do			15.8	15.1				
Mean							15.0	19.4	167.9	74.9	289.5	56.5
St. Mary:												
318	1928	Baldwin	P. O. J. 234	Plant cane	5.1	78.3	4.2	77.4			251.0	62.3
262	1928	Cypremort	do	do	10.5	55.3	15.6	16.1				
273	1928	do	do	do	11.3	51.9	12.7	31.7				
Mean					9.0	61.7	10.8	41.9			251.0	62.3

TABLE 5.—*Greenhouse inoculation experiments on inbred corn and sugarcane comparing 215 cultures of Pythium arrhenomanes from various localities in the Louisiana sugar district*—Continued

Parish and culture No.	Year isolated	Source of culture			Lancaster Surecrop corn				Louisiana Purple sugarcane			
					Experiment 63		Experiment 67		Experiment 71		Experiment 84	
		Farm or locality	Variety	Crop	Mean weight	Reduction from controls	Mean weight	Reduction from controls	Mean weight	Reduction from controls	Mean weight	Reduction from controls
					Grams	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
St. Mary—Continued.												
1484	1935	Bellevue	C. P. 28/19	Plant cane			16.2	12.9				
1485	1935	do	do	do			17.8	4.3				
1486	1935	do	do	do			16.6	10.8				
1517	1935	do	C. P. 807	Second ratoon			13.5	27.4				
1518	1935	do	do	do			10.5	43.5				
1519	1935	do	do	do			13.8	25.8				
1520	1935	do	do	do			12.5	32.8				
Mean							14.4	22.6				
1493	1935	Oak Bluff	Co. 281	Plant cane			14.5	22.0				
1494	1935	do	do	do			14.8	20.4				
1495	1935	do	do	do			12.1	34.9				
1496	1935	do	do	do			15.8	15.1				
1497	1935	do	do	do			12.2	34.4				
1498	1935	do	do	do			11.5	38.2				
1499	1935	do	do	do			14.2	23.7				
1500	1935	do	do	do			11.5	38.2				
1513	1935	do	do	do			12.2	34.4				
1514	1935	do	do	do			9.3	50.0				
1515	1935	do	do	do			11.9	36.0				
1516	1935	do	do	do			11.1	40.3				
Mean							12.6	32.3				
Terrebonne:												
11	1927	Southdown	P. O. J. 234	Plant cane	23.5	0	16.5	11.3				
39	1927	do	D-74	do	14.8	37.0	13.2	29.0				
40	1927	do	do	do	13.6	42.1	13.3	28.5				
42	1927	do	do	do	11.7	50.2	10.2	45.2				
50	1927	do	do	do	12.6	46.4	13.1	29.6				
58	1927	do	Louisiana Striped	First ratoon	22.0	6.4	15.6	16.1				
61	1927	do	do	do	12.0	48.9	12.0	35.5				

233	1928	Southdown	P. O. J. 213	Plant cane	22.0	6.4	17.6	5.4				
236-1	1928	do	do	do	25.9	+10.2	18.2	2.2				
730	1928	do	do	do	20.0	14.9	18.6	0				
780	1928	do	do	do	9.6	59.1	11.5	38.2				
1103	1931	do	P. O. J. 234	do	11.7	50.2	11.5	38.2				
Mean					16.6	29.4	14.3	23.1				
1452	1935	Southdown	Co. 290	Plant cane	11.1	52.8						
1453	1935	do	do	do	9.8	58.3						
1454	1935	do	do	do	11.2	52.3						
1455	1935	do	do	do	9.9	57.9						
1456	1935	do	do	do			9.1	51.1				
1457	1935	do	do	do			11.3	39.2				
1458	1935	do	do	do			10.0	46.2				
1459	1935	do	do	do			12.4	33.3				
1460	1935	do	do	do	9.2	60.9						
1461	1935	do	do	do			10.6	43.0				
1462	1935	do	do	do	8.8	62.6						
1463	1935	do	do	do	8.7	63.0						
1464	1935	do	do	do	9.6	59.1						
1465	1935	do	do	do			10.4	44.1				
1466	1935	do	C. P. 28/19	do	7.3	68.9						
1467	1935	do	do	do	11.4	51.5						
1471	1935	do	C. P. 807	do			8.2	55.9				
1472	1935	do	do	do			11.1	40.3				
1473	1935	do	do	do			11.5	38.2				
1474	1935	do	do	do	7.0	70.2						
1475	1935	do	do	do	8.0	66.0						
Mean					9.3	60.4	10.5	43.5				
1141	1930	Crescent Farm	P. O. J. 213	Plant cane	12.8	45.5	14.5	22.0	280.2	58.2		
1143	1930	do	do	do	9.4	60.0	12.0	35.5	205.8	69.3		
1144	1930	do	do	do	16.9	28.1	12.3	33.9	211.8	68.4		
1147	1930	do	do	do	10.8	54.0	12.8	31.2	223.0	66.7		
Mean					12.5	46.8	12.9	30.6	230.2	65.6		
1400	1935	Crescent Farm	Co. 290	Plant cane	9.0	61.7						
1401	1935	do	do	do	9.2	60.9			142.2	78.8		
1402	1935	do	do	do			8.6	53.8				
1403	1935	do	do	do			13.1	29.6	92.0	86.3		
1404	1935	do	do	do	7.3	68.9						
1405	1935	do	do	do			15.4	17.2				
1406	1935	do	do	do					75.6	88.7		
1407	1935	do	do	do	8.7	63.0						
1409	1935	do	do	do	11.2	52.3			114.3	82.9		
1410	1935	do	do	do	7.7	67.2						

TABLE 5.—*Greenhouse inoculation experiments on inbred corn and sugarcane comparing 215 cultures of Pythium arrhenomanes from various localities in the Louisiana sugar district*—Continued

Parish and culture No.	Year isolated	Source of culture: Farm or locality	Source of culture: Variety	Source of culture: Crop	Lancaster Surecrop corn: Experiment 63: Mean weight	Lancaster Surecrop corn: Experiment 63: Reduction from controls	Lancaster Surecrop corn: Experiment 67: Mean weight	Lancaster Surecrop corn: Experiment 67: Reduction from controls	Louisiana Purple sugarcane: Experiment 71: Mean weight	Louisiana Purple sugarcane: Experiment 71: Reduction from controls	Louisiana Purple sugarcane: Experiment 84: Mean weight	Louisiana Purple sugarcane: Experiment 84: Reduction from controls
					Grams	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
Terrebonne—Continued.												
1411	1935	Crescent Farm	Co. 290	Plant cane			12.2	34.4				
1412	1935	do	do	do	12.7	46.0						
1413	1935	do	do	do	10.5	55.3						
1414	1935	do	do	do			12.3	33.9				
Mean					9.5	59.6	12.3	33.9	106.0	84.2		
1468	1935	Ashland	C. P. 28/19	Plant cane	11.4	51.5						
1469	1935	do	do	do	7.1	69.8						
1470	1935	do	do	do	16.4	30.2	8.7	53.2				
1437	1935	Ranch	Co. 290	do	7.9	66.4						
1438	1935	do	do	do	6.7	71.5						
1439	1935	do	do	do			10.3	44.6				
1440	1935	do	do	do			10.5	43.5				
1441	1935	do	do	do	11.9	49.4						
1442	1935	do	do	do	12.8	45.5						
1443	1935	do	do	do	8.7	63.0						
1415	1935	Presquile	Co. 281	do	13.5	42.6						
1416	1935	do	do	do			13.2	29.0				
1417	1935	do	do	do	13.9	40.9						
1418	1935	do	do	do			18.2	2.2				
Mean					11.0	53.2	12.2	34.4				
West Baton Rouge:												
319	1928	Lobdell	Louisiana Purple	Plant cane	12.8	45.5	15.2	18.3				
797	1930	Catherine	P. O. J. 36	do	9.2	60.9	13.1	29.6			329.7	50.5
803	1930	do	do	do	17.4	26.0	16.4	11.8	354.5	47.1	457.1	31.4
804	1930	do	do	do	10.0	57.4	13.8	25.8	90.4	86.5	304.0	54.4
806	1930	do	do	do	14.4	38.7	13.8	25.8	284.4	57.5	278.9	58.1
815	1930	Cinclare	P. O. J. 234	do	12.9	45.1	13.3	28.5				
816	1930	do	do	do	15.2	35.3	13.4	28.0			339.1	49.1
Mean					13.1	44.3	14.1	24.2	243.1	63.7	341.8	48.7

1638	1936	Cinclare	C. P. 807	First ratoon							263.5	60.4
1640	1936	do	do	do							256.7	61.5
1642	1936	do	do	do							242.5	63.6
1644	1936	do	do	do							170.5	74.4
1646	1936	do	do	do							259.5	61.0
1648	1936	do	do	do							294.9	55.7
Mean											247.9	62.8
1491	1935	Poplar Grove	Co. 281	First ratoon			14.1	24.2	275.3	58.9		
1492	1935	do	do	do			13.5	27.4				
1526	1935	do	do	Plant cane			17.1	8.1				
1527	1935	do	do	do			10.9	41.4	153.7	77.0		
1528	1935	do	do	do			15.9	14.5				
1529	1935	do	do	do			14.1	24.2	140.5	79.0		
Mean							14.3	23.1	189.8	71.7		

COMPARISONS ON SUGARCANE

The primary object of isolate-group tests on sugarcane was to make specific comparisons between the first and second survey collections from the same or adjoining plantations for possible indications of a change in virulence of the fungus following the introduction of new, and often more resistant, varieties.

The very susceptible Louisiana Purple variety, formerly extensively grown in Louisiana, was used as the test host. Previously sprouted young mosaic-free plants of uniform size were transplanted to 3-gallon glazed crocks of quartz sand and watered with a nutrient solution, as described in a previous paper (*80*). The size of container and space required for satisfactory development of the plants limited the number of cultures that could be compared in a given experiment to less than half those afforded in the same greenhouse space when corn was used as the test host.

EXPERIMENTS 71 AND 84

The small lots of cultures obtained in 1927–31 (first survey) from each of five widely separated plantations were compared with an equal number selected at random from a large collection obtained in 1935 and 1936 (second survey) from mostly the same plantations. Second-survey cultures from three additional plantations from which earlier cultures were not available were included for comparison. These eight plantations are considered representative of large areas of sugarcane in the major subdivisions of the Louisiana sugar district. Naturally, this does not imply that their *Pythium* populations are necessarily also representative.

In both experiments inoculations were made in duplicate only, and the paired plantation groups were distributed in randomized arrangement on eight greenhouse slat benches with one uninoculated control crock on each bench. Experiment 71 extended from April 24 to July 3, 1936, and experiment 84 from September 30, 1936, to January 5, 1937. Mean green weights of plants and percentage reductions due to root rot are given in table 5 and discussed under the following headings in conjunction with the data from corn.

LOCALITY DIFFERENCES IN VIRULENCE

In table 5 the 215 isolates used in one or more of the four tests are classified according to the parish (county), year, plantation (farm), or locality, variety, and crop occupying the field at time of collection. The number of fields sampled and the number of isolates included in these tests differ widely for the different parishes. Some important parishes are not represented at all. As mentioned previously, no systematic survey was attempted. Therefore, while the collections are doubtless representative only of the individual fields from which they were obtained, they are not necessarily representative of the parishes, but are so grouped for convenience of discussion.

For visual comparison of parish or locality differences in virulence, the isolates tested in experiments 63 and 67 are segregated by survey and listed according to percent reduction of yield in the frequency graphs in figure 8. Since the two surveys were separated by several years, they are discussed separately here but compared directly in

the following section (p. 38). The number of isolates of the first survey are too few for several of the parishes to afford reliable locality comparisons.

It may be observed in figure 8, for experiment 63, that the first-survey group from Pointe Coupee is more virulent than comparable groups from Lafourche, Terrebonne, and West Baton Rouge, but milder than the mean of the three isolates from St. Mary Parish.

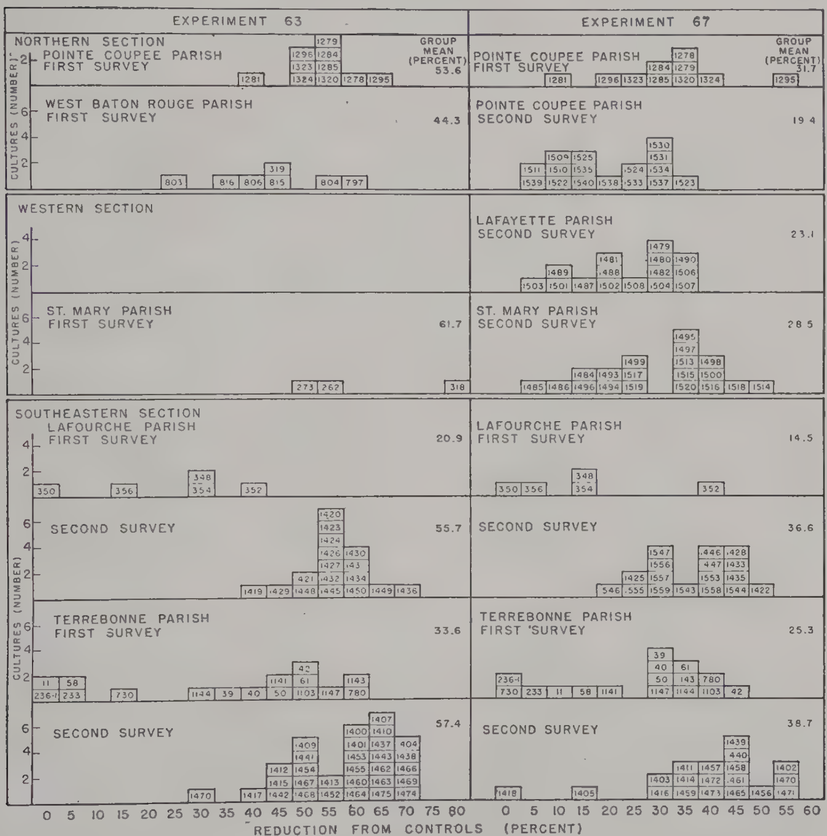


FIGURE 8.—Comparative virulence of groups of isolates of *Pythium arrhenomanes* from two surveys conducted in various representative localities of the Louisiana sugar district. (Limits of frequency classes extend 0.6 to 0.5 percent. Numbers correspond to isolate numbers in table 5.)

When the standard deviation of 3.44 g for individual isolate means based on 10 replications in experiment 63 is adjusted for the parish-group means in figure 8, it is found that all the first-survey groups plotted in figure 8 from this experiment differ significantly (odds of 99:1) from each other. However, this is not true in experiment 67 on almost the same first-survey collections. The generally less severe root rot in that test may have been responsible for the relatively smaller interparish differences. However, the trend is the same, i. e., Pointe Coupee is most severe, followed by Terrebonne and Lafourche.

The generally larger groups of the second survey afford more reliable determination of locality differences. In figure 8 (experiment 67) the groups from St. Mary, Lafourche, and Terrebonne extend noticeably further into the high percentage reduction classes at the right than those from Pointe Coupee and Lafayette. The parish means along the right margin show that the former caused 10 to 20 percent greater reduction in growth than the latter. The reliability of these parish differences may be examined by calculating from the standard deviation of 3.75 g for individual isolate means (based on seven replications), the percentage difference between groups required for odds of 99:1. On the basis of this standard, no significant differences are found between the northern parishes, Pointe Coupee and West Baton Rouge, and between these and the western parish, Lafayette; similarly, there was no significant difference between the southeastern parishes, Lafourche and Terrebonne, but the highly significant difference, as above indicated, is between the southeastern parishes (Lafourche and Terrebonne) and all other groups. The St. Mary isolates from typical Bayou Teche plantations occupy an intermediate position, definitely milder than the southeastern group but apparently more virulent (odds of 19:1) than the Lafayette group, which represents the southwestern section of the sugar district.

In view of the significant group differences found in these tests, it seems reasonable to conclude that the fungus actually differs in average virulence between certain of the fields or localities represented. Why this should be is not known. It is possible that its greater virulence in the southeastern section may represent adaptation to the highly resistant C. P. 807 variety, which for a number of years was grown much more extensively in this section than in other parts of the State.

INCREASE IN VIRULENCE FOLLOWING ADOPTION OF MORE RESISTANT VARIETIES

As mentioned earlier in this bulletin (p. 13), the isolates from the 1927–31 survey were obtained from the old noble varieties or the then recently introduced P. O. J. canes, while those of the 1935–36 survey were isolated after intensive culture of these canes and include fields of the subsequently introduced Co. 281 and the highly resistant C. P. 807 and Co. 290. From Greenwood plantation in Lafourche Parish and Crescent farm in Terrebonne Parish the *Pythium* was isolated from the same fields that had been sampled, respectively, 7 and 5 years previously.

The data in table 5 permit intraparish comparisons of 44 isolates from the first survey with separate groups (in the successive experiments), totalling 140 isolates from the second survey. These particular data are summarized in table 6 for more direct determination of differences between surveys. In the tests on corn, table 6 shows that the second-survey isolates from Lafourche and Terrebonne were significantly more virulent, and those from Point Coupee and St. Mary significantly less virulent than the isolates from the first survey, while the comparison for West Baton Rouge reveals no appreciable difference. The relative virulence of the larger groups is shown graphically in figure 8, where it is evident that, because of larger numbers, the fact that different second-survey isolates were used in

each test, and the agreement between tests, more confidence may be placed in the results for Lafourche and Terrebonne than for the other parishes of the sugar district.

TABLE 6.—*Differences in virulence between isolates of the 1927–31 (first survey) and the 1935–36 (second survey), as indicated by four inoculation experiments (summary of comparative data in table 5)*

[Comparisons in experiments 63 and 67 are between means of one or more localities, and in experiments 71 and 84 from a single plantation in each parish]

Parish and survey (source of cultures)	Comparative yields of inoculated plants in greenhouse tests on—							
	Lancaster Surecrop corn				Louisiana Purple sugarcane			
	Experiment 63		Experiment 67		Experiment 71		Experiment 84	
	Isolates tested	Mean weight	Isolates tested	Mean weight	Isolates tested	Mean weight	Isolates tested	Mean weight
	Number	*Grams*	*Number*	*Grams*	*Number*	*Grams*	*Number*	*Grams*
Assumption:								
First survey							3	247.0
Second survey							3	189.8
Percent difference (±) first survey								−23.2
Required for $P=0.05$								38.3
Lafourche:								
First survey	5	18.6	5	15.9	5	385.9	5	419.7
Second survey	17	10.4	17	11.8	11	150.2	5	256.4
Percent difference (±) first survey		−44.1		−25.8		−61.1		−38.9
Required for $P=0.05$		5.8		8.9		20.9		17.4
Pointe Coupee:								
First survey			10	12.7	7	187.7	3	285.9
Second survey			16	15.0	6	167.9	3	289.5
Percent difference (±) first survey				+18.1		−10.5		+1.3
Required for $P=0.05$				8.8		44.3		33.1
St. Mary:								
First survey			3	10.8				
Second survey			19	13.3				
Percent difference (±) first survey				+23.1				
Required for $P=0.05$				11.7				
Terrebonne:								
First survey	16	15.6	16	13.9	4	230.2		
Second survey	30	10.0	19	11.4	4	106.0		
Percent difference (±) first survey		−35.9		−18.0		−54.0		
Required for $P=0.05$		4.2		6.8		46.0		
West Baton Rouge:								
First survey			7	14.1	3	243.1	5	341.8
Second survey			6	14.3	3	189.8	6	247.9
Percent difference (±) first survey				+1.4		−21.9		−27.5
Required for $P=0.05$				11.0		50.4		20.5
Mean:								
First survey					19	257.6	16	337.9
Second survey					24	152.2	17	247.5
Percent difference (±) first survey						−40.9		−26.8
Required for $P=0.05$						17.8		11.9

The more specific comparisons on sugarcane of isolates from individual plantations (tests 71 and 84 in table 6) reveal, with but one exception, the greater virulence of the second-survey collections. The comparative weights of inoculated plants are presented graphically in figure 9, which in general confirms the results on corn (fig. 8) in showing large and consistent group differences between the surveys, particularly from the southeastern section of the sugar district. Fig-

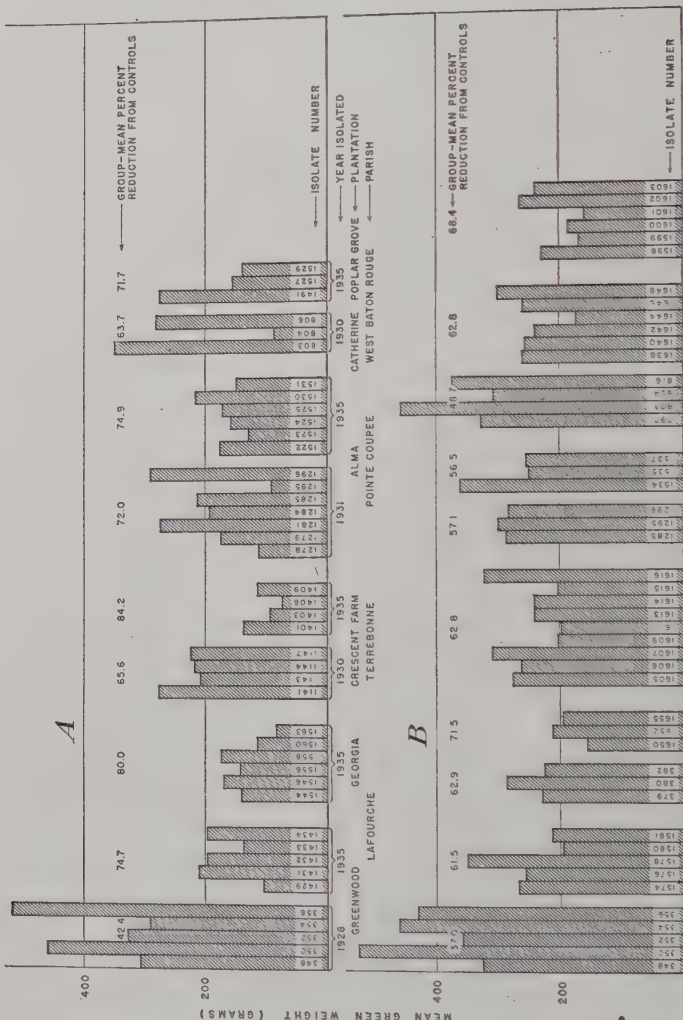


FIGURE 9.—Mean yield of plants of the very susceptible Louisiana Purple variety inoculated with isolates of *Pythium arrhenomanes* representing first- and second-survey collections from individual plantations. *A*, Results from experiment 71; *B*, from experiment 84. (Numbers correspond to isolate numbers in table 5.)

ure 10 illustrates typical differences in development of representative plants inoculated with first- and second-survey isolates from two plantations of this section. Although the first survey cultures permitted much greater growth than the second, when compared with the controls they also have rather seriously retarded the growth of this very susceptible Louisiana Purple variety.

The individual percentage differences naturally are subject to a large standard deviation because of the few replications. However,

since the number of isolates compared in these tests is approximately the same for each parish, general averages for each survey may be obtained and the differences with calculated errors reliably compared. Table 6 shows that the second-survey isolates averaged approximately 41 and 27 percent, respectively, more virulent than those of the first survey, and that such differences are statistically significant.

From the results of this preliminary study (which is being continued), it seems reasonable to conclude that during the 5 to 7 years separating the two surveys the average virulence of the fungus (as here represented) appreciably increased. This is conceivably due in part at least to a segregation and multiplication of certain biotypes

FIGURE 10.—Representative control and two pairs of inoculated plants from experiment 71, reflecting differences in severity of root rot caused by first- and second-survey isolates, respectively: *A*, Plant 1, uninoculated control; *B*, plants 2 and 3, inoculated with isolates from Greenwood plantation, No. 350 (26-percent reduction) compared with No. 1432 (70.3-percent reduction); *C*, plants 4 and 5 inoculated with isolates from Crescent Farm plantation, No. 1141 (58-percent reduction) compared with No. 1401 (78.8-percent reduction).

brought about by general adoption of more resistant varieties, which permitted survival of only the more virulent or adaptable components of the earlier population.

A possible alternative explanation of the differences between the two surveys would assume attenuation or loss of virulence by the first-survey isolates during prolonged maintenance in artificial culture. Edgerton, Tims, and Mills (*40*) report a gradual reduction in severity of root rot produced by one of their cultures which was tested repeatedly over a 3-year period. They interpret this as due to actual loss of virulence by the fungus. While no pronounced attenuation has become obvious in the writers' cultures, positive proof of a limited amount is obtainable with difficulty. Most reliable evidence might be expected from comparisons of relative virulence in infection tests

separated by a period of years. Therefore, pertinent data from four inoculation experiments on the same inbred line of corn have been summarized separately for this purpose.

Thirty-three isolates from the first survey were used in experiments 37 and 40, conducted between May 1932 and February 1933, and the results were published in a previous paper (*79*). The same isolates were again tested during the present study under identical technic in experiment 63 (September 17 to November 8, 1935) and experiment 67 (February to March 1936). There was thus a maximum period of approximately 3½ years between the two pairs of experiments. The average percentage reductions in weight of plants by these 33 isolates were in the separate experiments as follows: Test 37, 35.2; 40, 42.3; 63, 41.7; and 67, 25.8. Tests 40 and 63, which were conducted under temperatures and illumination more favorable for growth of the plants, show practically the same percentage of reduction. The weighted average of tests 37 and 40 is 39.2 percent and of 63 and 67, 36 percent. The difference is not statistically significant.

The values for individual isolates are plotted on the equiaxial diagram in figure 11, where it may be noted that those falling below the line of perfect agreement were more severe in the early tests (37 and 40) and those falling above, in the recent experiments (63 and 67). The actual numbers are 18 below and 15 above the line, and the percentages 54.5 and 45.5, respectively. The X^2 test gives odds of only 1:1 against agreement between the two pairs of experiments. Only four of the isolates (58, 236–1, 350, and 356) were strikingly more virulent in the earlier than in the recent tests, and their mildness in the latter possibly may have been due to attenuation. Nevertheless, when tested later in experiments 71 and 84 on sugarcane, Nos. 350 and 356 exhibited a considerable degree of virulence (table 5 and fig. 9). For most of the numbers, figure 11 shows that there was no significant change in virulence during the interval between the successive pairs of experiments. Therefore, the conclusion seems justified that the above-found differences between surveys were, in fact, due to greater average virulence of the second-survey isolates, which, as above stated, is most logically interpreted as an adaptation of the fungus to the newer varieties.

RELATION OF INCREASE IN VIRULENCE TO DECLINING YIELDS

If the shift toward greater virulence of the *Pythium* was in response to the general adoption of more resistant varieties, it is obviously of the greatest practical importance to ascertain what effect, if any, such shift has had on the yields of these varieties, especially after 5 to 10 years of intensive cultivation. This introduces the complex and controversial subject of so-called varietal deterioration and declining yields, which planters and scientists alike have discussed for many decades in most sugarcane-producing countries.

During the past 5 to 7 years in Louisiana there has been an unmistakable decline in yield of practically all the varieties introduced immediately following the failure of the old noble canes. P. O. J. 213 and P. O. J. 234 suffered widespread commercial failure from red rot and stubble deterioration, respectively, before their final replacement by more recent introductions. One of the latter, C. P. 807, has, in turn, had to be abandoned in most sections of the sugar district

because of red rot susceptibility. The increasing number of fields showing gappy stands and failing stubble crops of these three varieties became so obvious as to prompt the oft-repeated assertion by many experienced planters that apparently no new variety may be expected to maintain its yields longer than a short period of years.

Fortunately, during the past decade extensive agronomic and pathological data have been accumulated and now form a fairly reliable basis

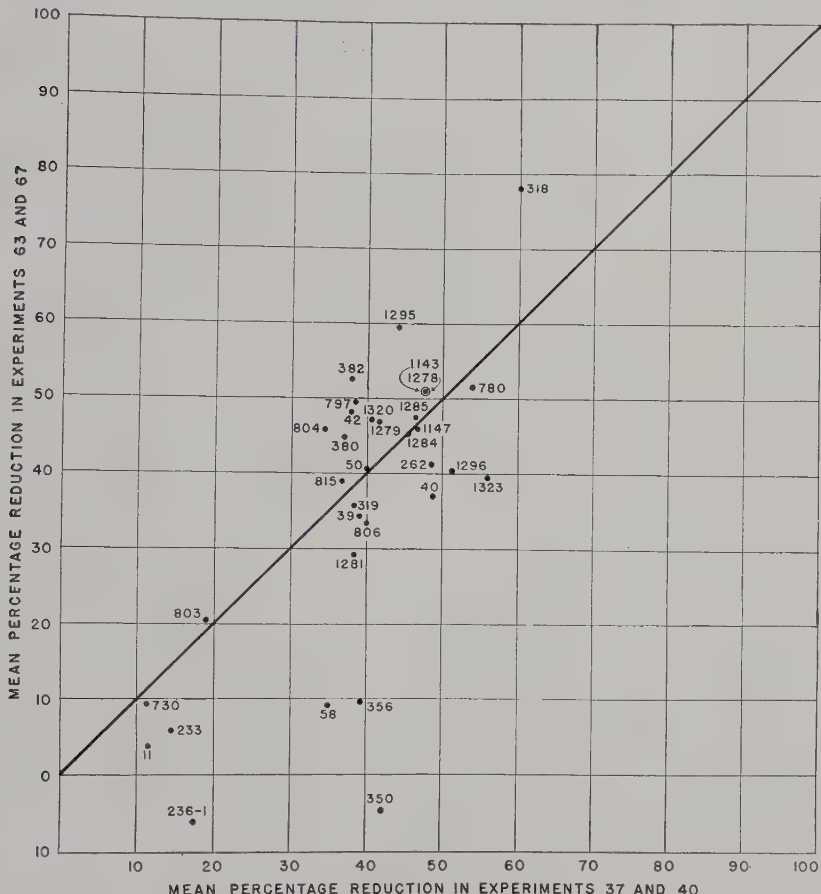


FIGURE 11.—Scatter-dot diagram comparing relative virulence on an inbred line of corn of 33 isolates of *Pythium arrhenomanes* before and after an approximately 3½-year period of storage in pure culture. (Numbers correspond to isolate numbers in table 5.)

for examining yield trends and their relation to disease severity, respectively. Replicated variety tests initiated in 1925 to 1928 by Rands and Sherwood (*81*) and Rands, Sherwood, and Stevens (*82*) on representative plantations in various sections of the Louisiana sugar district have since been continued and expanded by Arceneaux and associates, who have recently summarized (*11*, *12*, *13*) average annual yields from each of the varieties over a period of years. Their data for plant cane and first stubbles in the light-land tests during the period 1928–36 are shown graphically in figure 12. The plant cane

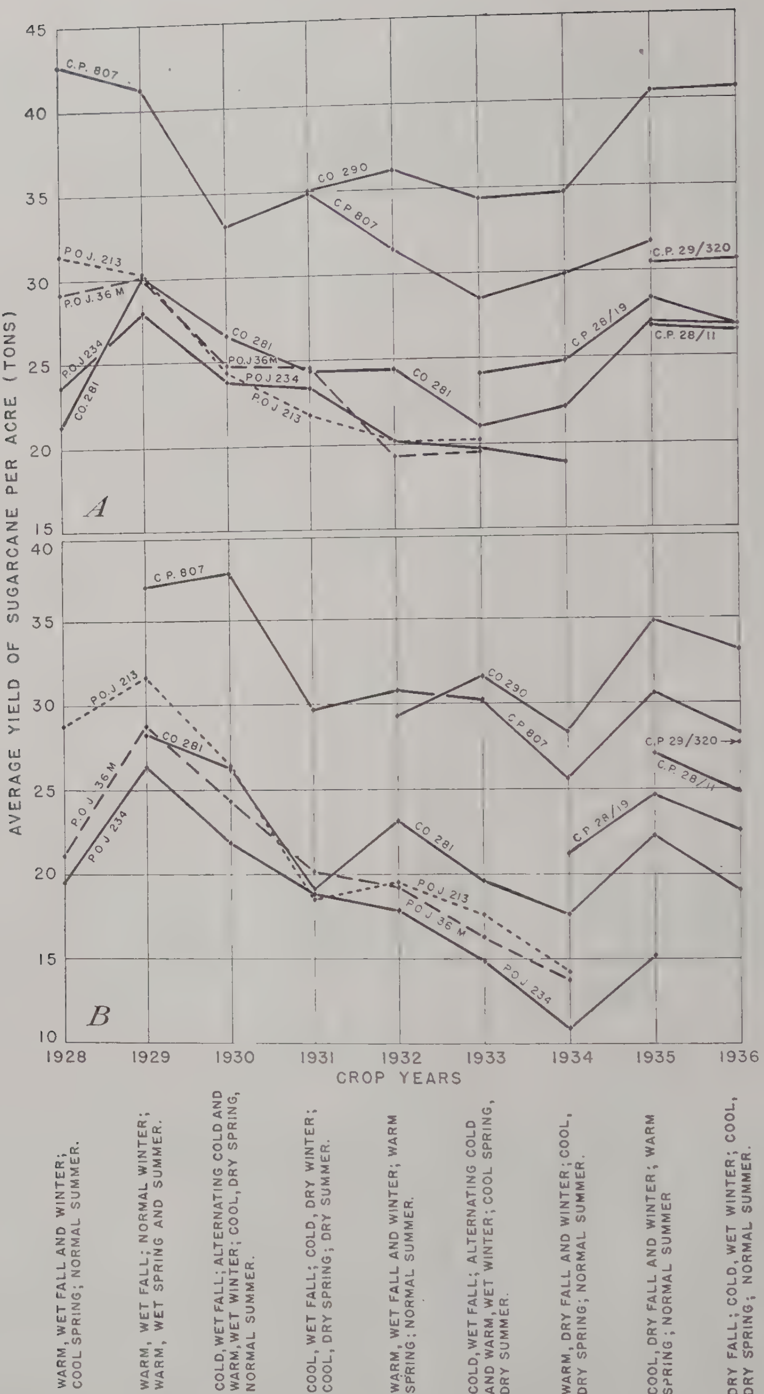


FIGURE 12.—Average annual yields of cane per acre obtained from important commerical varieties in 6 to 10 replicated yield tests located on light soils in representative sections of the Louisiana sugar district: *A*, Plant cane; *B*, first stubble. (Significant weather conditions are indicated for the crop year which begins in October of the preceding calendar year.)

yields of Co. 281 and C. P. 807 for 1928 are not strictly comparable with the remaining varieties, because they were not included in all tests on which these averages are based.

The downward trend of yields after 1929 of most of the varieties shown on this graph confirms popular opinion about such a trend for all of the earlier introductions. It is also reflected in the State-wide average yields shown in figure 1, although the decline did not reach any serious proportions, because shifts in relative acreage and increase of the newer introductions quickly restored and later increased average commercial yields.

Notes on the weather for each crop year at the bottom of figure 12 indicate that 1929 was probably most favorable of the entire period for growth of cane. However, 1932 and, to a lesser extent, 1935 were also favorable years, but the response by most of the older introductions continued in these tests was very much less than in 1928 and 1929. Therefore, the decline apparently could not have been due exclusively to direct or indirect effect of any temporary or prolonged period of unfavorable weather.

Up to about 1930 the land on which many of the tests were planted had been cropped previously only to the weaker-growing old varieties, and might, therefore, have been comparable to a certain extent with new or long-rested soil. Thus, the very high early yields in figure 12 reflect, to some extent at least, the apparent effect of temporary soil-fertility factors.

The investigations by Abbott (*2*, *4*) indicate strongly that shifts in population or development of new strains of the red rot fungus were responsible for the failure of P. O. J. 213 and severe damage to C. P. 807, while P. O. J. 36–M and Co. 290 have suffered intermittent injury. Of the widely cultivated early introductions there remain the P. O. J. 234 and Co. 281, both of which he considers resistant to red rot.

The Co. 281 and P. O. J. varieties shown in figure 12 are all moderately susceptible to root rot, while Co. 290 and C. P. 807 are highly resistant to this disease. Therefore, possible differences between yield trends of these latter two varieties and the susceptible Co. 281 and P. O. J. 234 would reflect less influence by red rot and might reveal some effect of the apparent increase in virulence of the root-rotting pythium. For more direct comparison, the average yields of the susceptible are expressed as percentages of the resistant varieties and the values for each year plotted in figure 13.

Co. 281 shows no prolonged directional trend for either plant cane or stubble when compared with C. P. 807, but reveals a noticeable drop in relation to Co. 290. Aside from differential response to weather conditions, both curves have doubtless been influenced to some extent by mosaic and red rot as well as root rot. In 1930 when Co. 281 was released no mosaic was reported in this variety, but by 1934 crops of this variety were found to be infected practically 100 percent by the mosaic disease; and, as above indicated, red rot is known to have increasingly damaged C. P. 807. Therefore, their yield curves have continued more or less parallel. Part of the decline of Co. 281 in relation to Co. 290 may have been due to root rot, but its magnitude and differentiation from the influence of mosaic cannot be determined.

The declining yields and eventual widespread failure of P. O. J. 234 suggests more clearly apparent increasing damage from root rot than from any other single causative factor. This tentative hypothesis

is based partly on negative evidence pertaining to the absence of important or progressive damage from other diseases and on many years of field study and observations of the variety. P. O. J. 234 has main-

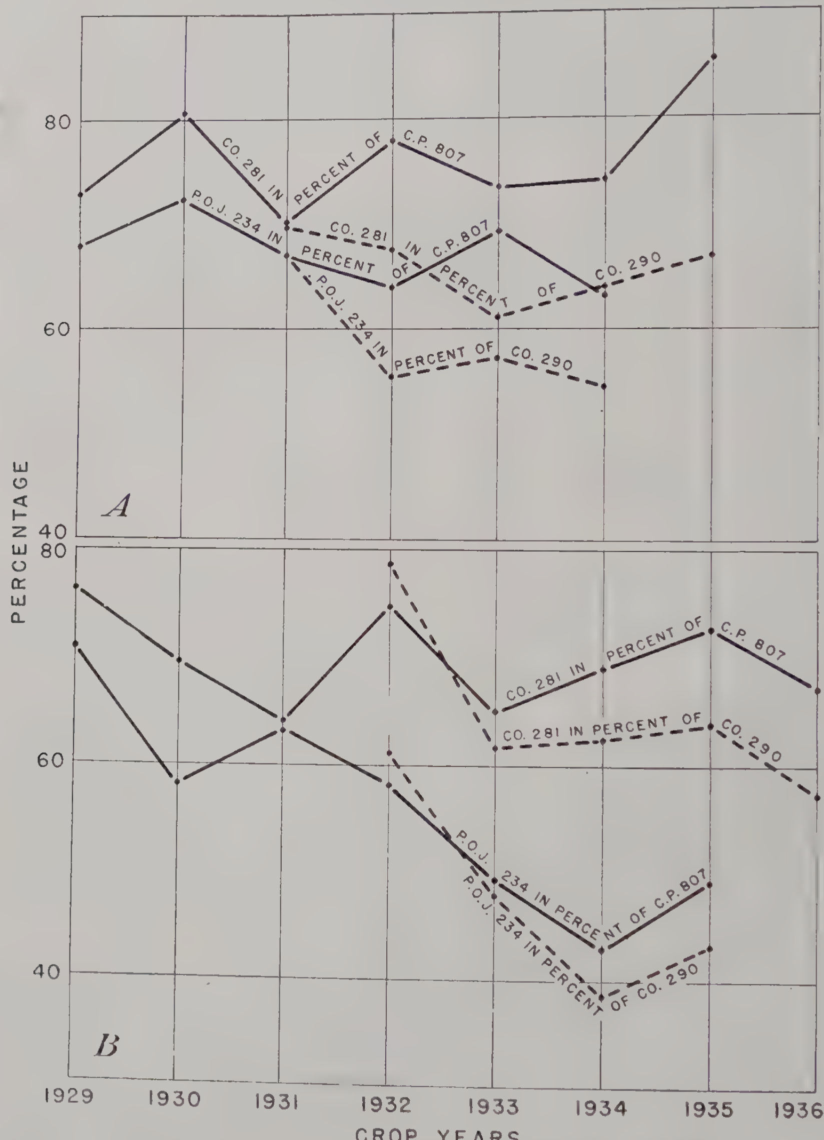


FIGURE 13.—Yield trends of root rot susceptible varieties in relation to resistant ones: *A*, Plant cane; *B*, first stubble. (Based on averages in fig. 12.)

tained a fairly high and constant percentage of mosaic throughout the period shown in figure 13. Comparative yield tests during the years 1930 to 1934 reported by Summers and Rands (*90*) show no evidence of a cumulative effect from planting mosaic-infected seed

cane; and, as above noted, the variety has maintained its resistance to red rot.

The commercial failure of P. O. J. 234 was popularly attributed to stubble failure or stubble deterioration, which means that at the beginning of the growing season a high percentage of the stubbles from the preceding crop failed to establish vigorous shoots or ratoons and furnish a satisfactory stand of stubble cane. Figure 13 shows that the greatest decline in relation to root rot-resistant varieties took place in stubble crops. The maximum difference between 1929 and 1934 was approximately 32 percent, compared with only 14 percent for plant cane.

Edgerton, Tims, and Mills (*41*) conducted extensive surveys of stubble deterioration to determine varietal resistance, the relation of weather, cultural practices, and the role of possible causative agents. They reached the conclusion that it may be caused or greatly influenced by a number of factors, which differ in severity from year to year on the same or on different varieties, such as continuously low as well as freezing temperatures, deficient soil aeration and drainage, maturity and time of harvesting the preceding crop, condition of stubble roots (including their destruction by root rot), the red rot fungus, stubble shaving, and other unfavorable cultural practices.

During a 5-year period stubble deterioration was found most severe in 1930 and 1934 and least injurious in 1929 and 1933. Among the earlier commercial varieties P. O. J. 213 and P. O. J. 234 were found most susceptible, while Co. 281, Co. 290, and P. O. J. 36 were most resistant. While the data on weather and stubble deterioration presented by these authors correlate very well with some of the extreme variations in yield shown in figures 12 and 13, their relation to the gradual decline in yield is not clearly apparent.

The writers' limited studies of the general stubble-deterioration problem confirm the above conclusions, but more detailed attention has been given to specific influence of root rot. Extensive field examinations in the spring of the P. O. J. varieties 36–M, 213, and 234, and the Co. 281 have revealed, with the passage of the years, what appeared to be unmistakable increase in severity of this disease. The trend has been emphasized by an increase in the number of fields showing backward growth of stubble crops both with and without preceding stubble deterioration. However, following the latter, root rot was always more severe on the young shoot roots. Such was particularly noticeable on P. O. J. 213 following damage to seed cuttings or stubble pieces by red rot. In case of P. O. J. 234 reduction of food reserves in the stubble pieces from sprouting and freezing back during the winter and early spring apparently weakens the old root system and hastens its deterioration. Many examinations indicated the formation of a new flush of rootlets on at least some of the old roots coincident with active sprouting and growth of shoots during warm periods of the early winter, and their subsequent destruction by root rot when growth was arrested or frozen back by low temperatures. Co. 290, a variety resistant to root rot, showed many healthy root tips and rootlets throughout the winter. Since all roots require elaborated foods, particularly carbohydrates, for their growth, the loss of functioning roots by the P. O. J. 234 resulted not only in a further drain on the stored reserves of the stubble, but con-

tributed to the physiological inactivity and eventual death of the latter when satisfactory conditions for growth were resumed in the spring.

In greenhouse experiments stubble failure due indirectly to root rot by *P. arrhenomanes* has been repeatedly produced. Nevertheless, under field conditions it is a complicated problem involving many factors as concluded by the above-mentioned investigators. Therefore, while the specific role of root rot as a factor in stubble failure remains to be determined, the apparently increasing prevalence of such failure, particularly in P. O. J. 234, supports the general field studies in suggesting that at least some of the decline in yield of root rot susceptible varieties has been due to specialization of *P. arrhenomanes.*

RELATION OF INCREASE OF VIRULENCE TO VARIETAL SUCCESSION AND PERMANENCE OF RESISTANCE

The evidence of physiologic specialization of the *Pythium* and its apparent influence on yields show that root rot must be looked upon as a dynamic rather than a static factor, as hitherto considered, in relation to sugarcane production. This altered point of view prompts the suggestion that possible changes in virulence of *Pythium arrhenomanes* may have been responsible for certain instances of declining yields, varietal failures, and substitutions in the past, examples of which have been cited by Earle (*35*) and in the historical section of this bulletin. A long succession of varieties has characterized the history of the sugarcane industry in many countries. In Java, for example, the partial succession, beginning about 1850, was White Japara, Black Cheribon (Louisiana Purple), P. O. J. 100, 247–B, E. K. 28, and the presently grown and moderately resistant P. O. J. varieties. The old Creole variety was followed successively in the West Indies by Otaheite, Crystalina, and various hybrids; and in Louisiana by Purple and Ribbon, D–74, and finally the smaller-stalked P. O. J. varieties, which, in turn, have recently given place to Co. and C. P. seedlings.

While probably in most instances the variety substituted was intrinsically superior to the one displaced, it is also true that such substitution was often necessary because of failing or increasingly uncertain yields of the older cane due to various diseases. Failures ascribed to root rot might naturally have resulted from accidental introduction and establishment of the causal fungus for the first time in the particular country. The original home or homes of *Pythium arrhenomanes* is not known. Therefore, it is necessary to differentiate between failures due to original introduction and spread of this, or whatever fungus may have been responsible, and subsequent possible changes in its virulence.

During the long history of root rot in Java, where the cause is still unknown, this disease has probably been mentioned more frequently in connection with varietal substitution than in any other country. Geerts (*48*) presents extensive data based on plantation yields showing a decline in production of P. O. J. 100, 247–B, and E. K. 28 during varying periods following their widespread cultivation. Houtman (*53*) gives production curves for E. K. 28, the

downward trend of which is roughly correlated with increase in percentage of area affected by root rot. Heilygers (*52*) and Kulescha (*59*) disagree with these authors that a permanent decline in yields had occurred. While certain of their curves also show a decline extending over as long a period as 10 years, this was often followed by periods of 1 to 3 years when production came back to the original level. Geerts' interpretation of declining yields and apparently increased susceptibility of varieties as due to degeneration or a permanent change having taken place in the phenotype of the variety itself was vigorously disputed by Bremer (*21*, *22*) and others.

Physiologic specialization of the pathogen would not necessarily cause a further decline in yield after an initial drop had been suffered from the development and spread of a more virulent strain, unless, of course, other and still more virulent strains should continue to appear. A state of more or less equilibrium between the new strain and its host might be expected with yields at a somewhat lower level, or they might be so affected at the outset as to represent occasional crop failure. The effect on the host conceivably might depend upon its particular degree of resistance toward the original population of the fungus as well as upon the extent of divergence of specialized strains of the latter. Accordingly, a wider divergence probably would be required to influence yields of a highly resistant variety than one only moderately resistant to the prevailing population of the fungus. Therefore, judging from the preliminary evidence presented in this bulletin, the greatest danger from physiologic specialization of *Pythium arrhenomanes* would appear to be in connection with the less resistant varieties, which thus far unfortunately have predominated among the many new seedling selections otherwise most promising for commercial use.

It is not known whether after prolonged culture the present resistant varieties such as Co. 290, C. P. 28/11, and C. P. 29/116 may go down in yield because of root rot. While in most years considerable rootlet-tip rotting has been observed, in no case could the effect on growth be considered important. A change in the present favorable situation with respect to these varieties will probably depend upon the potential amplitude of variation of the *Pythium*, which is not known, or upon possible changes in soil or other conditions favoring the disease. Of significance is the fact brought out later in temperature experiment K, that the highly virulent isolate 1323 is capable of damaging Co. 290 seriously. However, it is not yet known whether this isolate represents merely a chance variant or a specialized subpopulation of more virulent forms. If the latter should be found, a decrease in the present resistance of the variety might eventually be anticipated.

INFLUENCE OF ENVIRONMENTAL FACTORS

WEATHER IN GENERAL

Of the many environmental factors influencing the severity of root rot in Louisiana, weather is undoubtedly the most important as well as the most variable. Low winter and spring temperatures in con-

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junction with excessive rainfall on poorly drained and aerated soils are generally considered to represent the optimum environmental combination for maximum spread and damage by root rot. The usual influence of these factors on seasonal development and expression of above-ground symptoms is indicated in the description of the disease in an earlier section of this bulletin. Variation from year to year in amount and distribution of rainfall during critical seasons is responsible for many of the variations in severity of root rot. Striking differences have been noted even between adjacent rows when the only difference in treatment was a compacting rain during the week's interval separating their planting. That planted after the rain suffered much more severely, due apparently to less advancement of the crop before the onset of unfavorable winter weather. In other seasons no difference, or even the reverse effect, has been observed, depending entirely upon weather conditions between the time of planting and resumption of active growth in the spring.

A specific and less frequent weather combination that brought about such severe root rot damage to C. P. 28/19 in the spring of 1937 has been described in detail by Rands and Abbott (*77*). Stimulation of root and top growth during an unusually warm January followed by freezes and cold, wet weather in February and March was indirectly responsible that year for the root and seed-piece deterioration resulting in practical crop failure of many plant-cane fields of this susceptible variety on mixed and heavy soils.

Unfavorable winter and spring weather assume such importance in Louisiana because sugarcane, a tropical plant without a dormant stage, is being grown for a considerable part of the year in an essentially temperate climate. The minimum temperature for growth of roots and shoots is reported by Ryker and Edgerton (*85*) to be approximately 54° F., although Sartoris (*86*) found sprouting of roots and buds at as low as 42.8°. A number of investigators report growth to be exceedingly slow at temperatures little below 70°. Above 70° there is a progressive increase in growth with rise in temperatures to an optimum of 88° to 97°. This is illustrated in figure 14 for two P. O. J. varieties compared in greenhouse experiment C, photographed 1 month after planting in the constant soil-temperature apparatus described on page 53. At the two lowest temperatures (65° and 72°), P. O. J. 234 sprouted and grew more rapidly than P. O. J. 213, thus confirming under controlled conditions a widely recognized inherent difference observed by growers when these varieties were extensively cultivated in Louisiana. While maximum weight of plants of these hybrid seedlings, as also of noble varieties in other tests, was at 91° to 95°, the slightly smaller plants at 82° to 86° were invariably better proportioned and more typical of field-grown sugarcane.

The cardinal temperatures for growth of the *Pythium* in culture are reported in a previous paper (*79*) to be approximately 6° C. (42.8° F.) as the minimum, 30° C. (86° F.) the optimum, and 36° to 38° C. (96.8° to 100.4° F.) the maximum. While the optimum for the fungus may be slightly below that for the sugarcane plant, the range endured by both is similar.

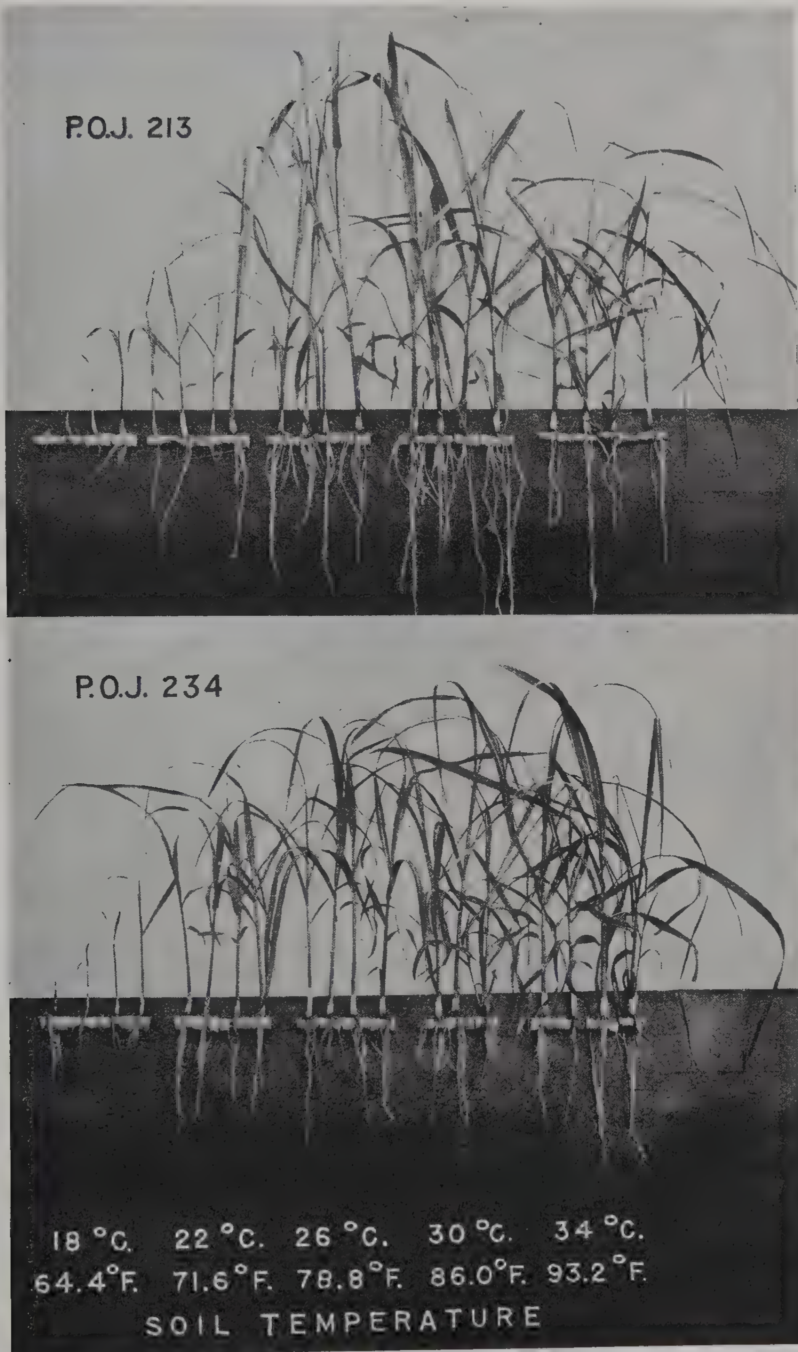

Figure 14.—Influence of temperature on rapidity of germination and growth of two P. O. J. varieties at five constant soil temperatures. The three-joint seed cuttings were trimmed for photographing.

A 5-year average soil temperature curve obtained from thermograph records at Houma, La., is shown in figure 15. When this is interpreted on the basis of the above temperature requirements of the crop, it is evident that the effective growing season extends on the average only from April to about mid-October. Therefore, although planting of most of the crop takes place in late September and early October, the initiation of rapid and uninterrupted growth must await the following April. A variable amount of sprouting and growth occurs immediately after planting and during warm spells in the winter, but this is often checked by subsequent cold and, in occasional winter seasons, is repeatedly frosted down to the ground.

Edgerton, Tims, and Mills (*41*) express the opinion that extended periods with temperatures from 40° to 42° F., which are common during Louisiana winters, tend to lower the vitality of both roots and stalks of sugarcane. The vicissitudes of the so-called dormant season

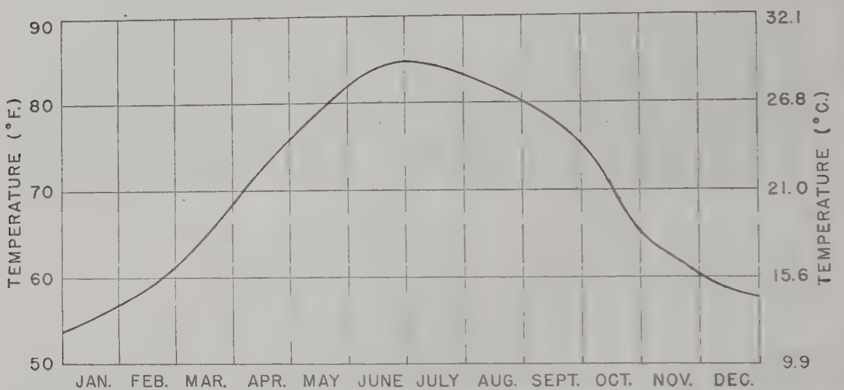

FIGURE 15.—Five-year average temperature of soil (Sharkey clay) at a depth of 3 inches at the United States Sugar Plant Field Station, Houma, La., for 1928–33. (Smoothed curve based on 3-day average of maximum and minimum recorded on soil thermograph records.)

not only render the plants vulnerable to disease but have a direct influence on eventual yield. McDonald (*62*) made an extensive analysis of weather and production records of the old varieties formerly grown in Louisiana and showed that high yields generally followed dry winters (January, February, and March), with often above-normal temperatures especially in March. Conversely, years with low average yield were characterized by wet winters with low temperatures in February and March. As previously mentioned in this bulletin, the old varieties are highly susceptible to both root rot and red rot, so it seems probable, as McDonald acknowledges, that a considerable part of such weather influence was due to an indirect effect on disease intensity. However, the same but less striking relationships are evident for the hardier varieties (fig. 12) that supplanted those on which the above study was based. The curves in figure 12 reflect, of course, both winter and summer weather, but it is well known that the root rot-resistant varieties Co. 290 and C. P. 807, when not damaged by red rot, have usually shown less influence from winter weather conditions than the susceptible sorts.

RELATION OF TEMPERATURE TO INFECTION AND DAMAGE BY PYTHIUM ARRHENOMANES

METHODS

The specific influence of temperature on infection and root damage by *Pythium arrhenomanes* has been studied in the greenhouse by means of controlled soil temperature tanks of the Wisconsin type. Ten insulated tanks measuring 30 by 36 inches and 24 inches deep were set up in two rows on each side of a power shaft to which was attached a screw propeller operating near the bottom of each tank to keep the water in motion and at a uniform temperature. For temperatures above that of the greenhouse, electric heating coils controlled by thermostats were employed, and for lower temperatures ice water from a refrigerating plant in the head-house basement was run into the tanks through an insulated pipe, the rate of flow being controlled by thermostats and appropriate solenoid valves. Thus any graded series of constant water temperatures between about 35° and 100° F. could be maintained automatically with an accuracy of approximately one-half degree.

Four watertight cans, 12 inches in diameter by 14 inches deep and holding about 55 pounds of moist soil, were suspended through holes in the top of each tank. Local clay loam with enrichment of well-rotted compost was placed in shallow trays and steamed twice under 5-pound pressure with a 2-day interval, remixed, made up to approximately 70 percent of its water-holding capacity, and apportioned to the cans. Subsequent watering to maintain uniformity was determined by periodic weighings. Previous experiments of this type on corn by Johann et al. (*56*) showed that a high moisture content (70 percent of the moisture-holding capacity) greatly favored infection by *Pythium arrhenomanes*.

Three to five three-joint cuttings were planted at a depth of 2 inches in each can, and the surface insulated by addition of 1½ inches of pulverized cork. The *Pythium* in pure culture on corn-meal-sand medium was introduced into the soil at the time of planting of the inoculated series. Usually four inoculated and four control cans were used at each temperature. After germination, all but two to four of the most uniform plants in each can were thinned out. Even with the most careful selection of seed cuttings and in thinning, and the use of two tanks (eight cans) for each temperature, there was still such variation in size of plants that necessarily large calculated errors were apparently inevitable in any quantitative comparisons. However, since only the soil temperature (and not the air surrounding the plants) was controlled, chief emphasis has been placed on qualitative or observational evidence on the effect of temperature on root development and activity of the fungus.

EXPERIMENTAL RESULTS

During the period 1928–36, 10 experiments of various types, running from 2 to 3 months each, were conducted with the equipment described in the preceding paragraphs. The earlier tests were mainly varietal comparisons between the very susceptible D–74 and Louisiana Purple and the more vigorous P. O. J. varieties 36–M, 213, and 234, conducted as an aid to interpretation of field behavior. Some

of the results were briefly summarized in 1929 by the senior author (74). Since these varieties are no longer commercially important and the influence of temperature on their infection by the *Pythium* was in all essentials similar to that of Co. 281, the earlier experiments may be summarized following presentation of quantitative results from two recent tests on the latter variety.

EXPERIMENTS J AND K

In experiment J an attempt was made to imitate field conditions under which summer or early fall-planted cane attains considerable growth unhampered by root rot until the onset of low temperatures of the so-called dormant season. Therefore, single-joint cuttings of Co. 281 were planted in 4-inch pots of steamed soil and sprouted in incubators at 36° C. (96.8° F.); after 9 days' further growth at favorble greenhouse temperatures, a series of uniform plants, 10 to 12 inches tall, was selected and reset in the cans with four plants per can, inoculated where required, and the cans returned to the tanks, which had been adjusted and were maintained at a wide range of constant soil temperatures. There were two inoculated and two control cans at each temperature.

In experiment K the temperature cans were planted directly with four three-joint cuttings, having the end buds gouged out, according to the standard procedure, and later thinned to two plants per can. There were two inoculated and two control cans at each temperature. Co. 281 was compared at five selected temperatures with the more vigorous and resistant Co. 290. The virulent strain No. 58 of *Pythium arrhenomanes* was used in experiment J; and the highly virulent No. 1323, in experiment K. The former test extended from April 14 to June 18, 1934, and the latter from March 13 to May 28, 1936. In both experiments average air temperatures were favorable for growth, ranging from 75° to 85° F., and daily illumination approached the maximum duration for the year.

On the eighth day after planting experiment K, one to four sprouts per can of both varieties at the 32° C. (89.6° F.) temperature were showing above the surface of the 3½-inch layer of soil and cork covering the seed pieces; 2 days later plants were numerous at 29° C. (84.2° F.) and beginning to appear at 26° C. (78.8° F.) while none showed before the fourteenth day at 23° C. (73.4° F.) and the sixteenth day at 20° C. (68.0° F.). They came up sooner and were more numerous in the controls, and at the time of thinning (sixteenth day) the comparative ratios of control to inoculated plants growing from eight seed cuttings at the various temperatures may be expressed as fractions, as follows:

	Co. 281	*Co. 290*
20° C.	3/0	6/0
23° C.	7/4	8/6
26° C.	8/5	6/7
29° C.	8/6	7/6
32° C.	7/7	8/8

At 20° and 23° the Co. 281 controls were a little slower than the Co. 290 in coming up, but no difference was noted at the higher temperatures. However, inoculated plants of the former were noticeably slower than the latter more resistant variety at all but the 32° temperature.

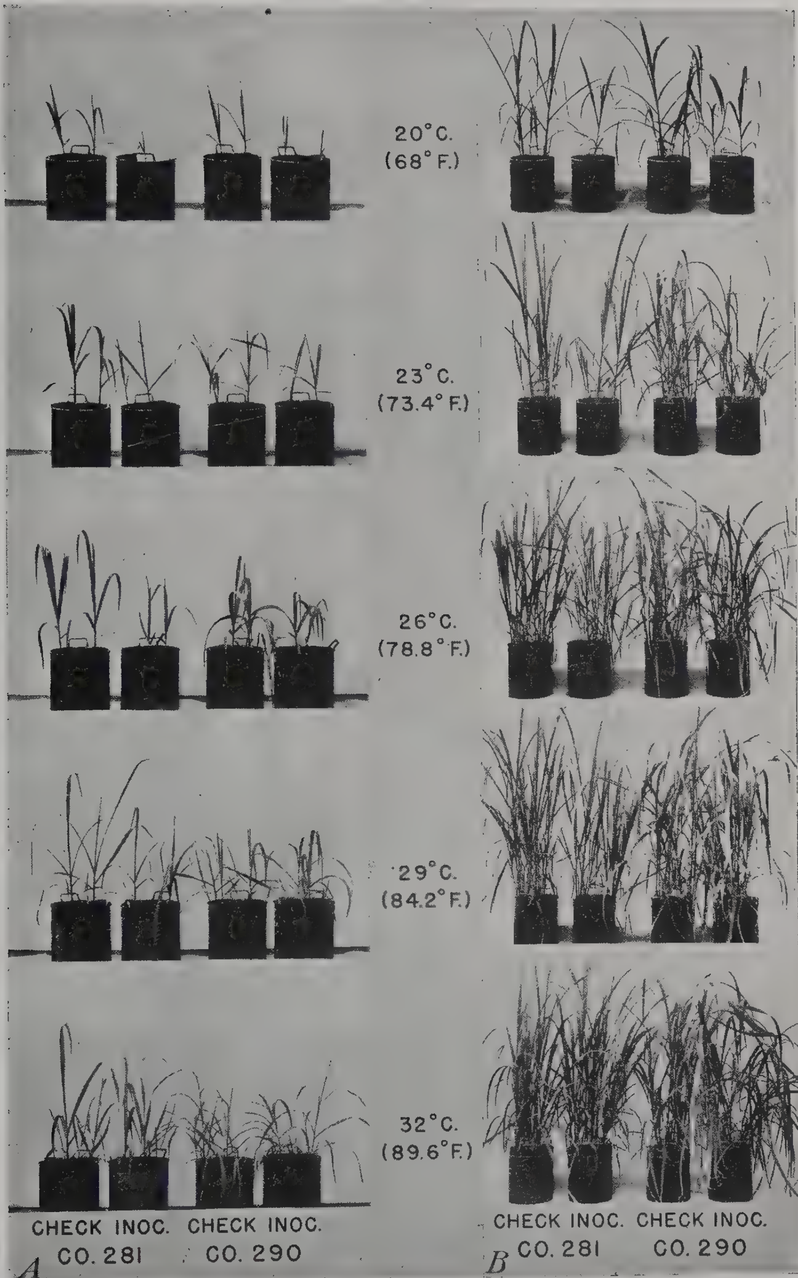

Figure 16.—Comparative growth after 18 days (*A*) and approximately 2½ months (*B*) of representative inoculated and control plants of two sugarcane varieties planted at five constant soil temperatures in experiment K.

Figure 16 illustrates comparative growth of representative control and inoculated plants of each variety at the five constant soil temperatures in experiment K when photographed 18 days (*A* series)

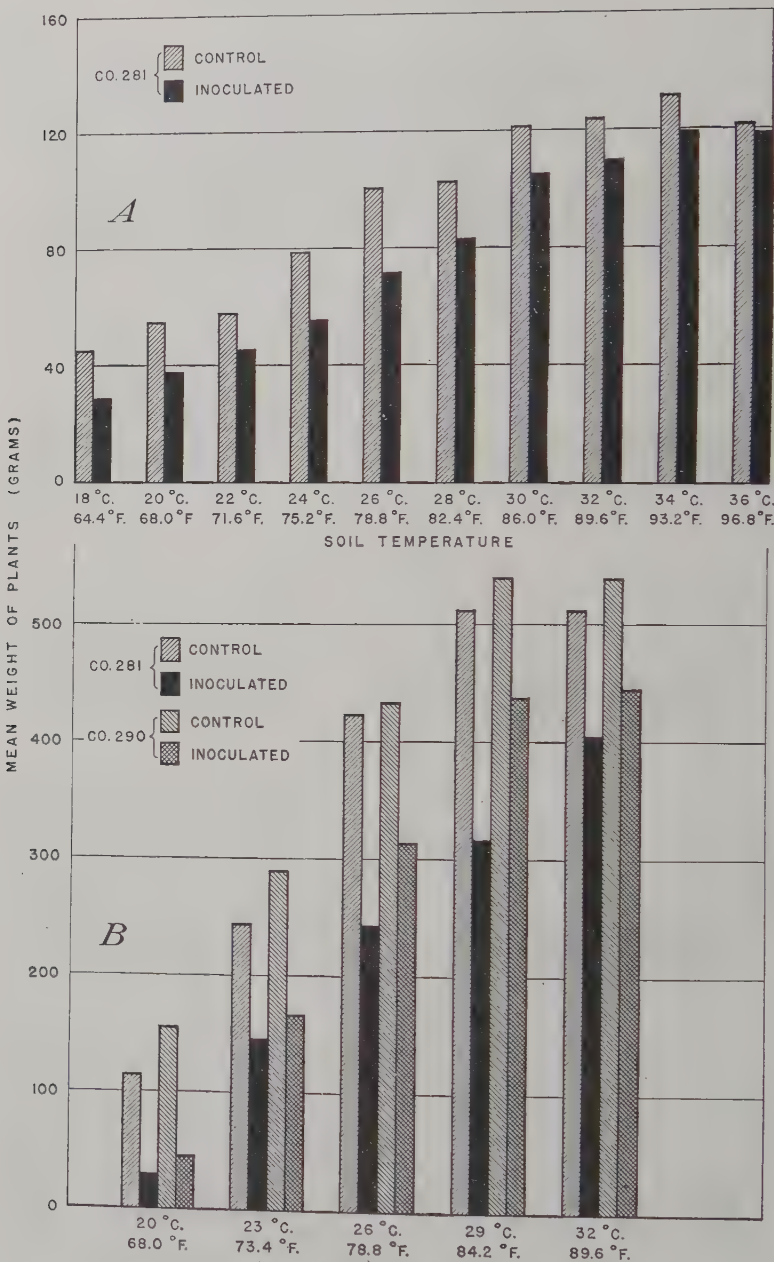

FIGURE 17.—Influence of soil temperature on growth of sugarcane and severity of root rot caused by *Pythium arrhenomanes* in two greenhouse experiments: *A*, Experiment J on Co. 281; *B*, experiment K on Co. 281 and Co. 290.

and approximately 2½ months (*B* series), respectively, following planting. The inoculated plants of both varieties showed, particularly during the early period, serious retardation of germination and establishment of the young plants on their own root systems. This

was most striking at the three lowest temperatures, which correspond, roughly, with Louisiana soil temperatures (fig. 15) from March to the middle of May. While otherwise apparently normal, the initial delay and consequent smaller size of such root-rotted plants persisted to the end of the experiment. Here the same soil temperatures were maintained throughout, but it is the general experience of planters that backward growth of the crop in the spring due to root rot or other causes is never compensated by subsequent favorable growing weather, and this fact in conjunction with a short growing season makes root rot of so much greater importance in temperate than in strictly tropical regions.

Average green weights per plant, cut off at the soil surface, in experiments J and K, are given in table 7 and shown graphically in figure 17. The optimum temperature for growth is seen to be between 32° and 36° C. (approximately 89° to 97° F.), which is in close agreement with that found by previous workers. Total growth was less in experiment J than in K, due to shorter duration of the test, and some competition for light, due to the use of four instead of two plants per can. Root rot was also much less severe in the former because of the employment of a less virulent but representative strain of the *Pythium* and the fact that the plants had been sprouted at optimum temperature and had developed a good root system before resetting and exposure to the fungus in the cans. Therefore, to a limited extent, experiment J was representative of summer-planted field cane that usually suffers less from root rot than the fall-planted crop imitated by experiment K.

TABLE 7.—*Results from two greenhouse experiments showing the influence of different soil temperatures on growth of sugarcane and the severity of root rot produced by Pythium arrhenomanes*

EXPERIMENT J, ON PLANTS PREVIOUSLY SPROUTED AT OPTIMUM TEMPERATURE

Temperature (° C.)	Variety	Mean weight per plant (including tillers)				
		Controls	Inoculated	Reduction due to inoculation		
				Actual	Required for $P=0.05$	Reduction
		Grams	*Grams*	*Grams*	*Grams*	*Percent*
18	Co. 281	45.3	28.7	16.6	16	36.6
20	do	54.5	37.9	16.6	12	30.5
22	do	58.0	45.7	12.3	17	21.2
24	do	78.4	55.7	22.7	16	29.0
26	do	100.5	71.7	28.8	30	28.7
28	do	102.2	82.3	19.9	21	19.5
30	do	120.6	105.2	15.4	29	12.8
32	do	123.7	109.5	14.2	36	11.5
34	do	131.0	119.7	11.3	33	8.6
36	do	121.2	118.4	2.8	34	2.3

EXPERIMENT K, ON PLANTS SPROUTED AT THE RESPECTIVE TEMPERATURES

Temperature (° C.)	Variety	Controls	Inoculated	Actual	Required for $P=0.05$	Reduction
20	Co. 281	114.7	30.9	83.8	30	73.1
	Co. 290	155.1	45.8	109.3	72	70.5
23	Co. 281	244.9	146.0	98.9	81	40.4
	Co. 290	290.4	165.9	124.5	62	42.9
26	Co. 281	424.3	244.0	180.3	135	42.5
	Co. 290	434.6	314.1	120.5	133	27.7
29	Co. 281	513.7	316.9	196.8	132	38.3
	Co. 290	541.9	437.4	104.5	173	19.3
32	Co. 281	512.7	404.8	107.9	235	21.0
	Co. 290	540.0	445.8	94.2	37	17.4

In both tests root rot caused greatest damage at the lowest temperature, 18° and 20° C., respectively, at which growth of the uninoculated plants was exceedingly slow, and the disease became progressively less serious with increase in temperature to 36°, which is past the optimum for cane growth. While the optimum temperature for mycelial growth of the *Pythium* is about 30° C., the reduction due to root rot at this temperature was only about one-third to one-half as much as at 18° to 20° C., which are so unfavorable for the growth of the plant. Therefore, it would appear that injury from root rot is more dependent upon the influence of temperature on the host than upon the parasite.

These findings on the relation of temperature to severity of root rot of sugarcane are in substantial agreement with those obtained with the same species of *Pythium* and with corn as the host, reported in Wisconsin by Johann, Holbert, and Dickson (*56*) and in Louisiana by Flor (*44*, *45*). Flor used sugarcane strains of the fungus and made limited studies also on this host, which reacted similarly to corn in showing more severe infection under cool temperatures unfavorable for rapid growth of the plants. In the more tropical climate of Hawaii, Carpenter (*28*) recognized soil temperature as of significance to the degree of injury from root rot but of secondary importance to nutritional factors. In Saskatchewan, Vanterpool and Truscott (*98*) tested the effect of temperature on root rot of wheat, employing their variety of *Pythium arrhenomanes* isolated from cereal root rot. They found that the amount of damage increased directly with increase in temperature, from 12° to 31° C., which, it may be noted, is contrary to the trend with corn and sugarcane. However, when it is recalled that wheat is essentially a low-temperature plant, at least in the seedling stages, these results are seen to be in agreement in demonstrating greatest susceptibility at temperatures less favorable for vigorous development of the crop.

The principal difference between the resistant Co. 290 and the susceptible Co. 281 compared in experiment K was the greater ability of the former to withstand the disease at several degrees lower temperature than the latter. For example, the percent reduction of Co. 290 at 29° C. was less than half that at 23°, whereas in the case of Co. 281 there were no significant differences between the three temperatures (23°, 26°, and 29° C.). At the indicated soil temperatures Co. 290 exceeded Co. 281 in yield by the following approximate percentages:

	Control series (percent)	*Inoculated series (percent)*
20° C	35	48
23° C	19	4
26° C	2	29
29° C	5	38
32° C	5	10

The percentages are statistically significant ($P=0.05$) only for 20° in the control series and for 20° and 29° in the inoculated series. However, the trend of the figures suggests that if the experiment were repeated with a greater number of replications, Co. 290 might have proved not only more resistant but inherently more productive at all temperatures than Co. 281, even in the absence of the fungus.

Character of root rot produced by *Pythium arrhenomanes* at a constant soil temperature at 18° C. (65.4° F.), (*A*) contrasted with a control plant (*B*). P. O. J. 234 variety photographed 44 days following planting. (Photographed by J. F. Brewer.)

A, Terminal portions of infected and healthy roots from 2-month-old plants of the Co. 281 variety grown at a constant soil temperature of 24° C. *B*, *Pythium* infected (*a*) and healthy (*b*) root systems of Co. 281 grown at a constant soil temperature of 24° C. The rotting and restriction of growth of the finer roots greatly reduces the root absorbing area and vigor of the plant. × 5. (Photographed by J. F. Brewer.)

in this moderately fertile garden soil. In this connection differences shown by replicated yield tests in Louisiana conducted by Arceneaux and associates (*11*, *12*, *13*), and reproduced in figure 12, are of interest as representing not only differences in susceptibility to root rot but the combined effect of all factors (including mosaic and red rot) differentially influencing these varieties in the field. The average yield of Co. 281 during the 6 years 1931–36 was 24.30 tons of plant cane per acre and that of Co. 290, 36.85 tons, which latter exceeds the former by 34.1 percent. The above temperature comparisons suggest that no inconsiderable part of this difference may be attributed to differences in severity of root rot.

ROOT-INFECTION STUDIES

Detailed examination and comparison of root systems in experiment K and B, C, and E of the earlier tests (not summarized above) revealed fairly consistent differences between low, medium, and high temperatures in character and extent of invasion by the fungus. At 15° C., all or a large proportion of the seed-cutting roots were killed soon after emergence, with often insufficient length having been attained to permit development of laterals. Due to paucity of functioning roots of this type, shoot roots have been noted to sprout from shoots less than 4 inches tall when normally they would not arise before the plant is 12 to 16 inches high. At 18° C. more roots of both types succeeded in growing, but later were severely tip-rotted, with most of the laterals killed back to their source. The typical appearance of inoculated and healthy plants of the P. O. J. 234 variety at this temperature in experiment B, photographed 44 days after planting, is illustrated in plate 3. The root condition is similar to that observed during March and April on fall-planted cane of this and similarly susceptible varieties in the field (fig. 3).

At intermediate temperatures of 20° to 24° C. progressively more roots became established, although still markedly deficient in mass with a brownish to blackish color which formed a striking contrast with the long, extensively branched, silky-white root systems of the controls. All of the younger large white roots showed reddish to dark-brown blotches or lesions from extensive cortex invasion, and the tips of many of the medium-sized, or secondary, roots were flaccid. Up to 80 percent of the tertiary roots or rootlets were tip-killed and often rotted back to their place of attachment, recognized by a blackish protuberance on the larger root. Plate 4, *A*, contrasts very young and older roots from inoculated and control plants of the Co. 281 variety grown at 24° in experiment E. Plate 4, ***B***, is an enlargement from roots similar to those shown in figure 4, and illustrates the typical and apparently harmless discoloration of older cortex, curtailment in growth of secondary roots, and particularly the pruning of rootlets, which latter is often responsible for extreme backwardness of early season growth of this variety in the field.

A characteristic feature of infected root systems at 24° C. and at lower temperatures was the number of individual roots that, apparently for awhile, escaped infection, made considerable growth, and then suddenly became severely rotted. Such, of course, may repre-

sent actual escape, but the circumstances often suggested that it might be due partly at least to temporary resistance connected with rapid growth. The very susceptible Louisiana Striped variety, for example, in experiment C, showed an initial very rapid and nearly complete germination of cutting roots that attained a length of 2 to 3 inches before being seriously attacked, whereas the resistant but very slowly sprouting Kassoer variety at 20° suffered almost complete loss of such roots at the time of their emergence.

At 26° to 30° C. the disease was more typically a rootlet rot varying from about 20 percent for Co. 281 and P. O. J. 234 to 80 to 90 percent of the rootlets infected for D–74 and other very susceptible varieties. The appearance of a D–74 root system grown at 30° is shown in plate 1, *A*, which illustrates the damage that may occur on such a susceptible variety even under midsummer soil temperatures.

At the high temperatures of 32° to 34° C. still more extensive whorls of cutting roots were permitted to develop and persist, although later these became severely tip rotted in the case of very susceptible varieties. Tip rotting of rootlets of the permanent root system was still sufficiently common to impart an amber-brown color to the root mass in contrast with the yellowish white of the controls.

At 36° to 39° C. the appearance of the inoculated roots was no different from the controls. However, on searching, a slight amount of cortex invasion and rootlet tip rotting could still be found, but the spread of the fungus following infection had evidently been quickly checked.

The *Pythium* was readily reisolated from infected roots over the entire range of temperatures employed. Microscopic examination of tissues revealed extensive production of lobulate sporangia and oospores at all temperatures in both the flaccid tissues of rootlets and firm or softened tissues of cortical lesions on large main roots. These findings in conjunction with prevalence of infections suggest that differences in soil temperature had little influence on the spread of the fungus through the soil, excepting a pronounced inhibition at 37° to 39° C. The total number of separate infections per plant, shown by the number of roots and rootlets attacked, was estimated to be much greater at temperatures of 26° to 34°, which are nearer optimum for both fungus and host than at lower temperatures where the root systems were smaller and the individual rotting more extensive.

The above summary shows clearly that the chief influence of temperature on disease severity was indirect through its effect on vigor and rapidity of growth of the plants. From a severe rotting of main roots at the unfavorably low temperatures, there was a gradual decrease in severity with increase in temperature to a mild rootlet pruning at temperatures near or above optimum for the plant. The effect of a more virulent strain of the *Pythium* was characterized by greater damage, particularly at intermediate and high temperatures, while the use of a more resistant variety tended to suppress the disease, particularly at these temperatures. Although such a generalization was valid for several experiments, it might not hold in all cases because of pronounced physiologic differences between strains of the fungus in reaction to temperature, as shown in a previous paper (*79*), and well-known differences in the temperature response of varieties of sugarcane.

SOIL CONDITIONS

Differences in the physical, chemical, and biological conditions of the soil undoubtedly exert an important direct or indirect influence on the severity of root rot. This is shown by the great variation in damage from field to field and in different parts of the same field, which has been noted by practically all investigators of the disease, and, of course, is also characteristic of root diseases of many other crops. Most of the conflicting opinions throughout the early literature, especially in Java, cite one or another soil condition as the sole cause of root rot. This was but natural since subsequent research has demonstrated that with very susceptible varieties a single soil factor, such, for example, as excessive nitrogen in Hawaii (*28*), may, in fact, be the sole indirect cause of a severe attack of the disease.

SOIL TYPES

In Louisiana, as in many other sugarcane regions, soil types characterized by a high percentage of clay give most trouble from root rot; usually the finer the texture the more severe is its effect, due largely, of course, to greater moisture-holding capacity, favoring the semi-aquatic mode of spread of the *Pythium*, and to poor drainage and aeration which are unfavorable for root growth. Such is typical of the extensive, low-lying areas of so-called mixed and black lands (Sharkey series) which, together, comprise from 20 to 40 percent of the average Louisiana sugar plantation. The higher lying and more porous sandy soils give generally much less trouble from root rot except in case of very susceptible varieties and unusual winter and spring weather conditions. The difference in this respect between typically sandy and heavy clay soils is usually so great that, as pointed out later, varieties are commonly classified as "light land" or "black land" canes, the former often being more or less susceptible and the latter resistant to root rot as well as to red rot and other unfavorable factors associated with such heavy soils.

The deleterious effect of poor drainage is widely recognized, but benefits from its improvement in Louisiana are generally much less than in countries having a pronounced dry season during which such heavy soils are thoroughly dried out, worked into a high state of tilth, and aerated to considerable depth. This probably directly reduces the *Pythium* population and, of course, greatly improves the chemical and biological conditions of the soil. Thus, following the severe drought of 1924 in Louisiana, noticeably improved crops of both sugarcane and corn were obtained for several years on these soils. Normally, however, they remain more or less continuously wet except for a thin surface layer and become practically saturated for days at a time during rainy winter periods.

TOXICITY

No local investigations have apparently been undertaken to determine the influence of periodic waterlogging and resultant deficient aeration on the chemical composition and possible formation and accumulation of reduced or toxic compounds in such root-rot soils. The possibilities have been discussed by Flor (*45*) and in a previous article by the writers (*80*). Flor subjected soil from a Mississippi

River seepage area to repeated washing, and, after drying and restoring to good tilth, compared the growth of plants in this with other lots of the same soil left untreated or treated with live steam for 2 hours. The superiority of plants in the steamed soil and similarity of growth in the washed and untreated portions suggested that soluble salts or toxins were not primarily concerned in the root-rot problem.

In the writers' article evidence was presented to show that if certain reduced organic compounds, even in dilute subtoxic concentrations, should be found in the poorly drained lands, such might readily account for the unusually severe root rot frequently encountered. Greenhouse sand-nutrient tests of salicylic aldehyde at concentrations of 20 to 40 p. p. m., which had little, if any, influence on cane growth in the absence of the *Pythium* and showed no effect on the latter in culture, apparently so predisposed the roots to infection that when the fungus was added plant-weight reductions were two to seven times greater than from the fungus alone. The same chemical enabled several mildly parasitic species of *Pythium* to cause significant damage to sugarcane, as shown on pages 21–25.

Vanterpool (*96*) demonstrated that species of *Pythium* causing browning root rot of cereals in Canada were themselves capable of producing a toxin which was thermostable and caused blackening of the seed and germination failure. The ability to produce toxins was not confined to parasitic species, and it is suggested that such may favor attack of the host by weak as well as strong parasites that might give some clue to the interrelations of the numerous saprophytic weakly and strongly parasitic forms of *Pythium* isolated from browning root-rotted plants.

Soil pH value, as determined for representative sections of the Louisiana sugar district by O'Neal and Breaux (*68*), is roughly associated with differences in root rot severity, but also with differences in drainage, so that any direct influence of the former is not readily determined from field evidence. The most acid soils (Lintonia series with pH 5.3) on the rolling lands near Lafayette are generally the best drained and have shown less root rot than the intermediate Yazoo and poorly drained Sharkey soils with pH 6.7 to 7.2.

In Hawaii, Carpenter (*28*) mentions excess calcium, magnesium, sodium, and the toxic salts of iron and aluminum as exerting a predisposing effect for root rot. In Java direct oxygen deficiency or toxic products arising therefrom has been most frequently mentioned as a cause, or causes, of root rot. From replies to a questionnaire sent to 100 plantations in 1928, Arrhenius (*14*) concluded that phosphate content of the soil, its pH value, evidence of reduction, and the moisture-holding capacity had no influence on the occurrence of root rot. On the contrary, low nitrate production and permeability, poor friability, and scouring appeared to be correlated with disease severity.

CONTENT AND AVAILABILITY OF SOIL NUTRIENTS

Ordinary fertility factors, such as pronounced mineral deficiencies or seriously unbalanced proportions of essential elements due to differences in availability, which are reported to be of major influence on sugarcane root rot in Hawaii (*28*, *29*, *64*) and in cereal root rot

in Canada (*97*), are, by virtue of their apparent absence, of little significance in Louisiana. One would not expect a highly unbalanced mineral content in an alluvial soil composed of sediments derived originally from the diverse geologic formations of 22 States. Therefore, nitrogen is the chief fertilizing element that must commonly be applied, with occasionally small supplements of phosphoric acid and potash.

Nitrogen in excess is known to aggravate diseases of many crops and particularly damping-off of seedlings caused by species of *Pythium*. Its general effect is to produce thinner cell walls that are presumably less resistant to fungus attack. From a recent review of the literature on nitrogen nutrition Allison and Ludwig (*8*) conclude that an abundant supply of soluble nitrogen causes greatly increased top growth but comparatively small root growth, due chiefly to lack of carbohydrates in the roots. These foods are first used in growth of tops, and a deficiency for the roots results. Therefore, the same amount of root rotting of a high nitrogen-fed plant would be more serious than on the larger root system of a low-nitrogen plant. Carpenter (*25*, *27*), in Hawaii, has demonstrated greatly increased severity of sugarcane root rot following heavy applications of nitrogen. Present-day commercial applications in that country are sufficiently high to bring on severe root rot and growth failure of the very susceptible Lahaina variety, but several times as much is required to break down the greater resistance of H–109. These findings suggested the hypothesis that the original widespread failure of Otaheite (Lahaina) might have been due to increased or acquired susceptibility to the *Pythium* brought on by increased—and for Otaheite, excessive—nitrogen fertilization which the plantations instituted around 1910.

In Louisiana a small increase in quantity of nitrogen has been applied to ratoons, and a general practice established of adding it to plant cane; both apparently without noticeable influence on root rot of susceptible varieties. Through cooperation with A. M. O'Neal, of the Division of Soil Fertility Investigations, Bureau of Plant Industry, the writers have had the opportunity of examining many fertilizer-rate tests for possible differential effect on root rot. Dosages of nitrogen alone in the form of ammonium sulphate and nitrate of soda applied individually or in combination at rates of 20 to 200 pounds per acre showed no significant differences in extent of root rot of the susceptible Co. 281 variety. However, most of the high rates of application had been made in the late spring on stubble cane so that subsequent high temperatures favored rapid root growth, which, with moderate availability of other essential elements in the soil, may have counteracted any serious predisposing effect of the treatment.

Increased root rot severity of plant cane due apparently to high nitrogen fertilization of the crop furnishing the seed has been repeatedly observed, but not experimentally proved by comparative tests correlated with chemical analyses of seed material. Nevertheless, a number of striking commercial field comparisons of plantings from heavily fertilized plots of new varieties being rapidly increased, or of Co. 281 from heavily fertilized mosaic-isolation plots, planted adjacent to field-run seed material have usually shown much greater winter root rot damage to the former. The situation was similar to tests of

cane tops compared with butts as planting material. Under favorable conditions the buds on the green tops which contain most of the nitrogen sprout quicker in the fall and make a better appearance, but they suffer more from winter root rot than plants arising from the maturer parts of the stalk. Therefore, while present commercial applications of nitrogen are apparently not aggravating root rot of the moderately susceptible varieties now grown, any large increase in dosage should take into account varietal susceptibility and the possibility of bringing about an unbalanced nutrition and increased damage by this disease.

The question of a possible insufficient availability of the so-called minor essential elements exerting a predisposing effect for root rot is naturally of importance when it is considered that many of the older lands in Louisiana have been in continuous cultivation (mostly with sugarcane) for more than a century. While records of field observations extending over the past 13 years have revealed no foliage symptoms or growth abnormalities that might be attributed to serious deficiency of any of these elements, several of them have been tested in replicated field experiments on sandy and heavy soils to determine any influence on root rot and cane yield. Boron at the rate of 250 pounds of borax per acre, copper in 160- and 320-pound rates of powdered bordeaux mixture per acre, manganese sulphate at the rate of 100 pounds per acre, and zinc sulphate at 20 and 60 pounds per acre were tested individually without other fertilizer treatment on plant cane of D–74, Louisiana Purple, or one or another of the less susceptible P. O. J. varieties. No application was repeated on succeeding stubble crops, but these received the regular fertilizer dressings, although not with purified salts. Results in all cases were entirely negative, both with respect to observable influence on root rot and measureable improvement in yield. Therefore, the details of the experiments may be omitted and the tentative conclusion drawn that the particular elements tested were already amply available in the soil of the experimental areas.

In peat soils of the Florida Everglades a combination of a naturally high nitrogen content and deficiency or unavailability of minor essential elements, notably copper, manganese, and zinc, has sometimes been associated with severe root rot of very susceptible varieties. From purely observational evidence it seemed that the deficiency itself was directly responsible for a large part of the curtailment of top and root development rather than an indirect effect by increasing root rot. However, in nutrient-deficiency experiments in Hawaii, Martin (*65*) and Carpenter (*28*) noted root rot that was apparently more severe than in the controls (complete nutrient) when each of several of the essential elements, excepting nitrogen, was omitted from the nutrient solution. Following standard fertilization with the above-mentioned elements in Florida, as recommended by Allison and associates (*9*), very susceptible varieties showed vastly improved root development and have been successfully grown on soils previously showing virtual crop failure.

ORGANIC MATTER AND BIOLOGICAL ACTIVITY

Biologically active soils with a moderate to high humus content usually produce crops with less damage from root rot than soils of similar type on which no effort has been made to maintain natural

fertility. In the absence of comparative soil studies no definite correlations are possible, but extensive observational evidence in relation to field histories indicate a definite influence of the biological activity of the soil on the disease. This is illustrated best by extreme cases where admittedly a whole complex of other factors may contribute to the observed effect. Such extremes are represented by severe root rot on occasional run-down and neglected fields of moderately heavy soil where legume crops (between cane occupations) have been cut for hay and no other organic materials excepting the cane stubbles and roots returned to the soil; and at the other extreme by certain soil types naturally very high in organic matter where the disease is particularly mild. Examples of the latter are a number of reclaimed muck areas in southern Louisiana and the "custard apple" peats of the Florida Everglades. Sugarcane has been grown for many years on these lands and *Pythium arrhenomanes* has been found to be well distributed in them, yet the very susceptible varieties D–74, D–95, E. K. 28, and Crystalina can still be grown successfully with usually no serious root-rot damage.

Less striking but more strictly comparable instances than the above, pointing to repeated applications of organic matter as the benefiting factor, have been observed on typical root-rot lands in Louisiana. For example, on Salsburg plantation in 1924, an area of D–74 was examined which showed much less damage from root rot and outstandingly better growth than nearby comparable fields. Inquiry revealed that during the preceding 7 years all field trash from both plant and stubble crops had been plowed under on the good area, but not on the others, and the surprisingly friable condition of the soil of the former indicated its great physical improvement by such treatment. Despite the difficulty in handling the more abundant trash of the newer varieties, several recent long-period commercial trials have been initiated. The older method illustrated by the senior author (*73*) has been discarded with adoption of heavy tractor-drawn plows, and supplementary nitrogen is sometimes used to hasten decomposition of the trash.

During recent years, similar contrasts in degree of root-rot damage and crop growth have been followed, involving the susceptible P. O. J. 234 and Co. 281 varieties planted on heavy soils with and without one or more prior applications of barnyard manure or filter-press cake. Because of susceptibility to root rot and other unfavorable factors in heavy soil, the Co. 281, particularly, is usually regarded as a strictly light-land cane, yet on these treated mixed and black-land fields, when also provided with good drainage, its growth and yields were sometimes equal to, or better than, those obtained on the light, front lands. Adjacent fields receiving only chemical fertilizers showed the backward growth characteristic of the variety on such soils. Problems connected with the use of filter press cake in Louisiana have been discussed by Rands (*75*). Heavy applications of the material have often resulted in low sugar yields, due to delayed maturity of the cane.

Beneficial effects of such organic amendments, including even blackstrap molasses, in improving growth on root-rot or growth-failure areas have been reported in several sugarcane countries. Indifferent and even harmful results were also occasionally obtained, so that no

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generalization is permissible because of differences in soils and other factors governing plant growth and disease prevalence. For this reason a detailed résumé of the literature may be omitted. However, nearly all investigators emphasize the value of such materials for increasing biological activity, which is usually notoriously low in cane soils due to the system of cropping. While greater vigor and resistance of the root systems from the improved physical condition of the soil, better aeration and drainage, and other known physical and chemical benefits from such applications may be solely responsible for reduction in root rot, direct biological effects on the fungus may also be important. The latter might kill off the *Pythium* or inhibit dispersal of its zoospores, liberated from infected roots, and also prevent possible spread of its hyphae through the soil. Since root rot is essentially a damping-off of the absorbing portions of the roots and rootlets, nearly every lesion represents a separate point of infection. Thus factors directly harmful to the *Pythium* would reduce the number of infections and consequently the severity of the disease.

Biological antagonism and actual antibiosis of fungus parasites have been shown to influence disease severity of a number of crop plants. A review of the general subject has recently been published by Waksman (*101*). Hartley (*51*) showed that damping-off of pine seedlings was less serious following simultaneous inoculations of saprophytic molds and the damping-off *Pythium* than when the latter was used alone. Tims (*91*) obtained a reduction in *Pythium* root rot of young sugarcane by adding cultures of an actinomycete to sterilized and previously inoculated soil in greenhouse tests. The actinomycete produced a toxin that inhibited growth of the *Pythium*. Striking antagonistic effects upon *Pythium* and *Rhizoctonia* have been demonstrated for species of *Trichoderma* by Weindling (*102*) and Haenseler and Allen (*49*) in studies of damping-off diseases of citrus and cucumbers, respectively. These were also attributed to production of toxic products, although microscopic evidence presented by the former author showed relationships suggesting that the *Trichoderma* exerted also a direct parasitic action on these fungi.

In a microbiological study of a sugarcane soil at the Louisiana Agricultural Experiment Station, Abbott (*1*) found that members of the genus *Trichoderma* ranked third in total numbers of fungi isolated. Their abundance was explained as possibly due to the considerable quantities of cane trash, which is largely cellulose, annually turned into the soil. Since *Trichoderma* is known to attack cellulose very vigorously, its characteristic green fructifications are commonly noted on pieces of dead leaves and stalks lying in the field. It is particularly favored by high soil moisture, and, under high temperatures, the writers have found it to be a serious obstruction to obtaining pure cultures of the root-rotting *Pythium*. Mixed isolations, as well as pure *Pythium* cultures later becoming contaminated with *Trichoderma* through mite infestation, quickly die.

This very incomplete summary of biological antagonism in relation to root parasites suggests that at least a part of the above-noted reduction in root rot following applications of organic matter to root-rot lands may have been due to the operation of this factor. Its greater effectiveness in the muck and peat soils should theoretically accompany the more intensive biological activity in such soils under the controlled drainage employed. This is indicated by lowering of

their elevation in certain instances as much as several inches per year from decomposition and settling.

Since filter-press cake is an abundant and highly organic waste product on at least all factory plantations, a cooperative study of its chemical and biological influences on root rot and sugarcane yield was arranged in 1932 with the Division of Soil Fertility Investigations. Chemical analyses and preliminary data on methods of handling the material were summarized by McKaig and Rands (*63*). The unfortunate loss of the chemical laboratory by fire and the transfer of personnel caused the discontinuance of the project after only a few preliminary field experiments had been conducted. These showed no outstanding benefits from ½- to 2-ton applications under plant cane and as a side dressing on stubbles. Maturity was delayed with often a net loss in recoverable sugar. Therefore, pending further experimental work, the present commercial practice of making 4- to 10-ton applications per acre on land being prepared for legumes which are grown between successive cane occupations would appear to be the safest procedure for disposal of the material and securing the soil and crop benefits that it gives.

INFLUENCE OF FARM PRACTICES

CROP ROTATION

Root rot is generally of little importance throughout the sirup-producing sections of Alabama, Florida, Georgia, and Mississippi apparently because of the long rotation of crops commonly followed. Under this system, long recommended by experiment stations for control of the root knot nematode and for maintaining soil fertility, the land is planted with other crops for a period of 3 to 5 years between successive cane occupations. When such crops are clean cultivated ones and are resistant to *Pythium arrhenomanes*, such as cotton, sweetpotatoes, peanuts, and other legumes, the *Pythium* largely dies out, as shown by many root examinations and isolation attempts. However, both the *Pythium* and nematodes are sometimes reintroduced by the unfortunate practice of planting the rootstocks along with seed cane taken from infested fields.

A similar influence of rotation is also evident under diversified farming along the northern and western fringe of the Louisiana sugar district where the cane is grown for either sirup or sugar production. Here, for many years after its abandonment in the sugar district proper and in the absence of serious red-rot damage, the very susceptible Louisiana Purple gave excellent plant-cane yields.

Typical "soil weariness" from maximum accumulation of root-rotting organisms is found under the plantation system of southern Louisiana, where economic, weather, and soil factors preclude crop diversification and length of rotation followed in the sirup districts. The rotation commonly practiced involving 1 year of legumes (often interplanted with corn) between successive cane occupations of the land has little or no influence on prevalence of root rot. A rotation practiced on many small farms in this section, involving 2 years of legumes or 1 year of legumes and 1 of truck crops, has given less root rot and generally greater yields of the moderately susceptible varieties of cane than under the regular plantation system.

The actual number of years that *Pythium arrhenomanes* can persist in the soil in the absence of sugarcane has not been determined. In air-dried soil in the laboratory it has been found to survive for more than 3 years, as proved by periodic planting of portions of the soil with disinfected cuttings of sugarcane and isolation of the fungus from the root rot that developed. Under field conditions it is known to attack corn and has also been isolated from occasional rotted root tips of various grasses (mainly species of *Syntherisma* and *Panicum*). While the fungus may survive over a period of years in the absence of sugarcane, as, for example, in pastures or abandoned fields, its population is very greatly reduced. Striking comparisons between regularly cropped and long rested land were noted on many sugar plantations during the restoration of the Louisiana industry. A recent instance, involving C. P. 28/19 on heavy soil, has been mentioned by Rands and Abbott (*77*).

One or more years of flooded rice crops have been noted in most, but not all, cases to reduce very greatly the *Pythium* population of cane land. Such seems paradoxical, because in unflooded greenhouse inoculation tests rice of the Blue Rose variety was severely root rotted by *Pythium arrhenomanes*. Apparently conditions arising from the flooding were responsible, but why they were not always effective remains unexplained. Most of the references to this subject in the Javanese literature indicate less root rot under a 3-year than a 2-year rotation of sugarcane, the former of which included two flooded rice crops and the latter only one.

TIME OF PLANTING

The very susceptible varieties formerly grown usually showed less root rot when planted during the period from November to February. This delayed most of the initial root growth until the onset of higher temperatures in the spring, which are less favorable for root rot. However, in some years, potential benefit from late planting in relation to root rot was more than offset by increased damage from red rot, which was favored by greater dormancy of the seed cane compared with early fall plantings.

With the varieties now grown, Co. 281 and C. P. 28/19, which are moderately susceptible to root rot, but more resistant than those formerly grown to red rot, November planting likewise gives generally less root rot damage than September and early October plantings. The latter plantings, during some winters, suffer severely from wide fluctuations in temperature with stimulation of growth followed by freezing back of the young plants, which doubtless weakens and predisposes them to root rot.

October planting of C. P. 28/19 on heavy soils is particularly hazardous, as recently pointed out by Rands and Abbott (*77*). On the other hand, early August planting of this and other varieties, which must go on the mixed and heavy soils, has resulted in least damage from both root rot and red rot, and has been demonstrated by Arceneaux (*10*) to yield cane of higher sugar content at harvest. Serious root rot is apparently prevented by establishing the young plants on their own root systems so that a degree of maturity and resistance comparable to stubble cane is attained before the onset of unfavorable winter weather. However, this was not effective for

P. O. J. 234 because of stubble deterioration and root rot. Therefore, summer planting may not be expected to have the same ameliorative effect on all susceptible varieties.

Because of the difficulties explained in the above-cited articles, only limited acreages of cane can, as a rule, be planted in August, and November planting frequently interferes with harvest operations. Nevertheless, where root rot-susceptible varieties must be grown on the heavier soils, preference should be given to these planting periods. The remaining acreage can be put in at the regular planting time from September 20 to October 15.

SEEDBED PREPARATION

A well-pulverized and settled seedbed is essential in obtaining a good stand of cane and maintaining a vigorous and healthy root system. It is often impossible to have such a bed, however, due to delay in turning under the legume crop, especially when it is grown with corn. Wet weather postpones gathering the corn, the soybeans are allowed to pass their optimum stage for soil improvement, they become very woody, and their coarse stems, together with the cornstalks, do not readily break down when finally plowed under. The result is a late-prepared, unweathered, loose, cloddy seedbed that may later permit serious drying of the seed cane and otherwise practically prohibit establishment of many of the temporary or seed roots.

Some of the worst cases of winter root rot have been observed under such conditions. In certain heavy-soil fields the damage was so severe as to suggest the operation of a temporary toxic condition connected with the mass of undecomposed refuse that directly inhibited growth or predisposed the roots to *Pythium* attack. Minute soil animals were also very destructive, apparently because the poorly pulverized soil permitted greater access to the sprouting seed roots. When the tips of these were eaten off before they were long enough to branch, a combination of such injury with root rot left the seed stalks with their sprouts nearly completely devoid of roots. The resulting physiologic inactivity of the cutting favored increased damage by seed-deterioration diseases, and these aided by root rot of the poorly established plants have often caused serious stand reductions and sometimes complete failure during late-spring dry periods.

While corn cannot ordinarily be omitted from all rotation land, the difficulties and losses frequently suffered by the succeeding cane crops, because of delay in preparation of heavy soils for planting, would justify either extending the rotation so that corn does not immediately precede cane or withholding its planting on such areas.

DEPTH OF COVERING

A 4- to 6-inch covering of soil over the fall-planted seed cane as applied by most plantations is probably the safest depth to use from the standpoint of both root rot and seed-deterioration diseases. Deeper planting often results in poor germination, as shown by Abbott (*3*), due to greater damage by both types of diseases, which are favored by the higher moisture and lower temperatures in the early spring surrounding such deeply buried seed before the excess dirt is removed. For the same reason, long delay in scraping or removing

excess dirt from more shallow-covered cane may increase root rot damage. If well pulverized, a thinner covering of heavy soil may be used than light sandy soil, which tends to wash more from the winter rains.

The thickness of the soil surrounding stubbles has a similar influence on early root rot of the ratoon crop as it does on plant cane. The earlier off-barring can be done without risk of freezes, usually the better the stand and less is the damage from root rot. Early off-barring hastens drying and warming-up of the compacted stubble ridge, which stimulates quick sprouting and rapid growth of the young ratoons. During warm, dry springs greatly delayed off-barring or complete omission of this expensive operation on light lands has resulted in no deleterious effect on yields; however, during cool, wet springs practical stand failures have sometimes been noted, especially with Co. 281, from such delay or omission.

Early fall harvesting followed by immediate sprouting of the stubbles and successive freezing during the winter greatly weakens and predisposes the new stand to root rot. This is particularly hazardous with quick-sprouting susceptible varieties like P. O. J. 234. However, as a rule fields to be carried through another crop are not harvested early.

VARIETAL RESISTANCE AND SUSCEPTIBILITY

FIELD METHODS

As outlined in a previous paper (*76*), imported varieties and agronomically promising seedling selections are tested in small field plots in an area of heavy soil known to be highly infested with the most virulent strains of *Pythium arrhenomanes*. Plots of control varieties of known reaction are interspersed among them for direct comparison and judgment of seasonal influence, which latter must be taken into account in comparing the reaction of varieties tested in different years. For the same reason the date of planting is approximately the same each year, i. e., October 1 to 15, when also many commercial fields are started. Great variation from year to year in winter and spring weather necessitates repetition of the tests if a particular season has not favored severe root rot of susceptible controls. In the meantime those seedlings eliminated on agronomic grounds need not be repeated. Those of greatest promise may be continued for several years or observed in agronomic experiments in order to determine as accurately as possible their particular degree of resistance or susceptibility to the disease.

The plantings are examined during the period from April to June, and both above- and below-ground symptoms of root rot, or their absence, are relied upon for estimating the degree of susceptibility. Extent of wilting shown by tight-rolled leaves and marginal yellowing during periods of dry weather, uneven size of plants, and lack of normal color and thriftiness are important above-ground symptoms reflecting root rot. However, the degree of susceptibility sometimes may be overestimated if seed-root failure has been due to early destruction of the seed piece by red rot, black rot, cytospora rot, or pineapple disease. Therefore, when a variety is not known to be resistant to these diseases, or following a bad red rot winter, exami-

nation of seed pieces and condition of their nodal roots and those arising from the young shoots is essential. Even then all field estimates of susceptibility necessarily are based not only on the extent of root deficiency caused by *Pythium arrhenomanes* but a varying amount of damage from other species of *Pythium*, as well as by the numerous minute, root-gnawing soil fauna.

RATINGS

For convenience all varieties are rated in increasing order of susceptibility from 1 to 6, since no immune variety has been found. Class 1 represents a high degree of resistance indicated by a majority of the seed and shoot roots that sprouted in the fall being able to survive the unfavorable winter and spring growing conditions. The plants ordinarily show little or no wilting even after several weeks' drought when growing in the heaviest clay soils. Examples of well-known varieties in this class are Co. 290, Kassoer, and Uba. In class 2 slight wilting may be noted on at least 5 to 15 percent of the plants and the roots show much tip rotting and partial loss of fine laterals. Examples are P. O. J. 2364 and C. P. 29/116. Classes 3 and 4 (intermediate) represent proportionately increased severity of the above symptoms. Examples of class 3 are P. O. J. 213, P. O. J. 2878, and C. P. 29/320, and of class 4, P. O. J. 36–M and P. O. J. 826. Class 5 (susceptible) and class 6 (very susceptible) show in plant cane severe wilting, yellowing, partial or extensive stand failure, and practically complete loss of roots during bad root rot seasons. First-stubble crops of the class 5 varieties may be better or worse than the plant cane, depending upon winter and spring weather conditions; those of class 6 usually show partial to complete growth failure on even the lightest and best drained land. Examples of class 5 are Co. 281, C. P. 28/19, and P. O. J. 234; and of class 6, most noble varieties of sugarcane.

RESULTS

STANDARD VARIETIES

A number of foreign and local varieties of importance in the breeding program, or of past or present commercial significance in Louisiana, are classified according to the above susceptibility ratings and listed under the following main groups according to their taxonomic affinity or derivation. The ratings are tentative in many cases, particularly of certain varieties in the intermediate classes 3 and 4, because they were not always tested during severe root rot years and in no case was any large number included during the same year.

1. Noble varieties (*Saccharum officinarum* L.):[7]
    - *Class 2.*—N. G. 106 (426).[8]
    - *Class 3.*—21 N. G. 2 and 5; N. G. 21, 80, 90, 97, and 106 (508).
    - *Class 4.*—B. H. 10/12.
    - *Class 5.*—H–109, H. Q. 5, 21 N. G. 15, S. C. 12/4.

[7] The varieties listed under *Saccharum officinarum* may include hybrids resulting from uncontrolled or natural crosses with other species of *Saccharum*.

[8] New Guinea varieties are classed tentatively as noble canes, although unpublished studies by G. B. Sartoris reveal evidence of remote admixture with *Saccharum spontaneum* in several of the numbers.

Class 6.—Badila, Creole, Crystalina, D–74, 95, and 625, D. I. 52, E. K. 2 and 28, Fiji, Hak-kwat-Che, Louisiana Purple and Striped, L–511, Manjav, New Guinea varieties (18 additional numbers of Brandes-Jeswiet collection), Otaheite, P. O. J. 100, Simpson, Striped Mauritius.

2. Indian canes (*Saccharum barberi* Jeswiet):
   - *Class 2.*—Hatooni.
   - *Class 4.*—Chunnee.

3. Chinese canes (*Saccharum sinense* Roxb.):
   - *Class 1.*—Kavengerie, Cayana, Chukche, Oshima, Tekcha, Uba, Yontanzan.
   - *Class 2.*—Khera, Kinar, Merthi.

4. Wild canes (*Saccharum spontaneum* L.):
   - *Class 1.*—Various introductions from India, the Malay Archipelago, and the Philippine Islands.

5. Wild cane (*Saccharum robustum* Jeswiet):
   - *Class 5.*—28 N. G. 251 (type collection from New Guinea).

6. Interspecific hybrids, F_1 selfs, and backcrosses:
   - (*a*) Group 1×2—
     - *Class 3.*—P. O. J. 36, 213, 501, 979, and 2379.
     - *Class 4.*—P. O. J. 36–M, 826, 1337, and 1499.
     - *Class 5.*—P. O. J. 228, 234, and 1335.
   - (*b*) Group 1×3—
     - *Class 1.*—C. H. 64/21.
   - (*c*) Group 1×4—
     - *Class 1.*—G–107, Kassoer, Toledo.
     - *Class 2.*—Hinds Special.
     - *Class 3.*—I–1081.
   - (*d*) Group 1×6, c:
     - *Class 2.*—P. O. J. 2364.
   - (*e*) Group 1×6, d:
     - *Class 3.*—P. O. J. 2722, 2725, 2727, 2878, and 2883.
     - *Class 4.*—P. O. J. 2714.

7. Complex hybrids with inheritance from groups 1, 2, and 4:
   - *Class 1.*—Co. 290, C. P. 807, C. P. 28/11.
   - *Class 2.*—C. P. 29/116.
   - *Class 3.*—C. P. 29/320.
   - *Class 4.*—P. O. J. 2753.
   - *Class 5.*—Co. 281, C. P. 28/19.

Most of the noble varieties (group 1) fall in the highly susceptible class 6, although within this class there are widely recognized differences. For example, the historic Creole variety, which was brought to the New World by Columbus, is by far the most susceptible, with Otaheite a close second, while D–74 and Crystalina are probably the least damaged.

The Indian canes (*Saccharum barberi*) here represented are slightly less resistant than the Chinese canes (*S. sinense*) and the wild sugarcane of Asia (*S. spontaneum*). Thus, interspecific hybrids between noble and Indian (group 6, *a*) are generally less resistant than those from Noble × Chinese and Noble × Wild Cane (group 6, *b* and *c*). During 1933 and 1934 some 200 F_1 seedlings from each of four crosses of D–95 (*S. officinarum*) × *S. spontaneum* (4 collections), and Otaheite by one of the latter collections, were all of equal resistance to Kassoer, a natural hybrid of Louisiana Purple × Glagah. On the other hand, it may be noted that I–1081 (Lahaina × Glagah) is considerably less resistant than Kassoer. In earlier years several hundred selfed seedlings of Kassoer were found to approach or equal

the latter in resistance, which was to be expected considering the absence of pronounced segregation of other characters.

Unlike the Noble × Indian hybrids, the F_1 seedlings from Noble × *Saccharum spontaneum* are rarely of direct commercial value, but require backcrossing to noble varieties, which apparently results in increased susceptibility to root rot, as shown by group 6, *d* and *e*. However, the numbers are obviously too few to permit any deductions on inheritance of resistance beyond the above-mentioned F_1 generations.

Practically all of the present-day commercial varieties in Louisiana are of extremely complex inheritance and are listed under group 7. Those in the first two classes only, i. e., Co. 290, C. P. 807, C. P. 28/11, and C. P. 29/116, are sufficiently resistant to root rot to be generally dependable for planting on mixed and heavy soils. However, as mentioned on page 42, C. P. 807 has already been abandoned in many parts of the sugar district because of too great susceptibility to red rot, which sometimes also reduces stands of the Co. 290 (*4*). The C. P. 29/320 (class 3) has not been seriously damaged by root rot, but its planting in heavy soils is considered hazardous by Abbott (*2*, *3*, *4*) because of red rot.

Co. 281 and C. P. 28/19 (class 5) succeed only in light land where their yields during many years are doubtless considerably reduced by root rot. On heavy soil these varieties usually are reduced in yield, and during bad root rot years, e. g., 1936–37, they may be very seriously affected, as illustrated in plate 2, *A*, and figure 18.

Because of its greater vigor, the C. P. 28/19 has been successful in August plantings on heavy soil, which attain a more advanced development than regular October plantings and tolerate the early season root rot injury. Such plantings largely escape severe seed-root destruction by soil fauna to which this variety seemed particularly attractive.

Edgerton, Tims, and Mills (*41*) have worked out a method for judging combined resistance to stubble deterioration factors, which includes root rot. Results obtained by these workers in connection with general disease tests at the Louisiana Station (*39*, *93*) on promising seedlings and commercial varieties showed P. O. J. 213 and P. O. J. 234 to be very susceptible and P. O. J. 36, P. O. J. 36–M, Co. 281, Co. 290, C. P. 807, C. P. 28/11, C. P. 28/19, and C. P. 29/320 to be highly resistant toward stubble deterioration factors.

FIGURE 18.—Comparison of resistant and susceptible varieties on heavy soil: *A*, Co. 290 (resistant) and Co. 281 (susceptible); *B*, C. P. 28/11 (resistant) and C. P. 28/19 (susceptible). Due to nearly complete loss of seed-cutting roots and initial shoot roots during the winter and early spring, plants of the susceptible varieties showed backward growth and leaves yellowed and tightly rolled when photographed during a dry spell in May.

SEEDLING SELECTIONS

Table 8 gives root-rot ratings on 111 seedlings representing first-year and later agronomic selections under test by George Arceneaux and associates at the United States Sugar Plant Field Station, Houma, La. Most of these are selections from among more than 5,000 seedlings from 26 crosses of the C. P. 1932–33 series which were produced by G. B. Sartoris at the Department's breeding station at Canal Point, Fla., and later tested as "complete" progenies by the pathologists at Houma. Data on these 26 seedling families, as well as those of the more recent 1934 series and the large number of primary selections that precede the agronomic selections and are determined largely on the basis of appearance and disease resistance, have been summarized in brief annual reports (*5*, *6*, *19*, *76*).

TABLE 8.—*Preliminary classification of certain agronomic selections, under test at Houma, La., according to their apparent resistance or susceptibility to root rot when grown in heavy soils*

Seedling No.	Root-rot class No.	Seedling No.	Root-rot class No.	Seedling No.	Root-rot class No.	Seedling No.	Root-rot class No.
C. P. 29/94	5	C. P. 32/182	2	C. P. 33/118	[1] 3	C. P. 33/307	4
99	3	202	5	121	4	310	1
103	4	206	5	122	2	329	2
120	2	209	4	125	3	342	[1] 3
137	3	286	2	136	4	359	3
142	[1] 1	298	[1] 1	138	5	365	4
C. P. 31/110	[1] 2	300	1	140	2	370	3
114	3	320	4	142	3	372	4
146	[1] 1	321	4	152	4	382	4
152	4	322	[1] 1	153	5	389	5
160	4	324	3	162	1	394	[1] 3
161	2	325	[1] 1	164	2	397	3
258	3	328	2	165	[1] 1	400	[1] 2
509	[1] 2	334	1	166	2	406	1
516	[1] 2	336	5	185	3	409	[1] 1
551	[1] 1	339	[1] 2	216	5	414	5
566	5	343	[1] 2	224	2	418	4
568	3	354	1	228	3	420	4
C. P. 32/2	[1] 2	C. P. 33/6	[1] 2	229	4	430	4
97	4	14	3	232	4	435	3
118	3	18	3	233	2	437	3
120	3	36	2	243	3	445	3
123	5	47	3	253	[1] 2	449	4
124	5	48	5	255	[1] 1	450	[1] 2
126	[1] 1	51	3	257	[1] 1	459	[1] 2
134	3	53	3	258	4	472	[1] 2
138	[1] 1	99	5	261	[1] 2	476	[1] 1
146	[1] 2	102	2	262	4		

Summary:	
Class 1 selections	19
Class 2 selections	28
Class 3 selections	28
Class 4 selections	22
Class 5 selections	14
	111

[1] Seedlings apparently combining sufficient vegetative vigor with the indicated root-rot resistance to recommend them for experimental agronomic trials on the heavy root-rot soils.

While the original nursery plantings suffice for preliminary estimates of the degree of resistance or susceptibility to mosaic and red rot, they are reliable only for eliminating seedlings falling in class 6 (very susceptible to root rot). This is because they must be planted on the lightest and best lands for maximum increase of planting material during the first year. In many instances such favorable conditions,

FIGURE 19.—Extreme difference in susceptibility to root rot between two seedlings from the same cross (C. P. 1933, L progeny) which became evident only in the first stubble crop (in 1936) here shown. (*a*) L–112 resistant class 2, and (*b*) L–39 (C. P. 33/352) very susceptible class 6, in first nursery planting on light soil. Because of favorable soil conditions and low incidence of root rot during the plant-cane crop of 1935, these seedlings were of equal vigor and size.

combined with spring planting and great varietal vigor, prevent elimination of class 6 seedlings as plant cane. However, they are readily detected in the succeeding stubble crop, as shown by the example in figure 19. The primary or pathology selections from the light-land nursery may be planted on heavy soil where the greater severity of root rot enables their finer classification. The best of these in agronomic qualities (i. e., the first-year agronomic selections), as, for example, the C. P. 33 series numbers in table 8, are usually replanted in root-rot tests to determine the reliability of their initial classification. Therefore, ratings on most of the 29, 31, and 32 series numbers in table 8 are more reliable than the 33 series, which are, for the most part, based on the single, although bad, root-rot season of 1937.

The totals at the bottom of table 8 show that nearly half of these selections have been placed tentatively, at least, in the resistant classes 1 and 2. Several of them possess also great vigor of growth, so that if their apparent resistance is maintained in further tests and they should also prove superior in agronomic qualities, they may be expected to reduce the hazards from root rot and to increase the yields in the Louisiana sugar district.

VIGOR IN RELATION TO RESISTANCE

Inherent vigor of growth apparently enables certain varieties to escape, or at least tolerate, a mild attack of root-rot, when less vigorous, but no more susceptible, sorts may be damaged seriously. In the former, rapidity of growth and root replacement seem to counteract the effect of the disease. This has been frequently illustrated in heavy soil comparisons of C. P. 28/19 and Co. 281, which are about equally susceptible. During seasons with mild root rot the C. P. 28/19, by virtue of its greater vigor and ability to grow at lower temperatures, has shown much better development than Co. 281, but during severe seasons it has been no better and sometimes much worse than the latter on heavy soils.

In order to determine the extent to which resistance or susceptibility of plant cane may be associated with differences in degree of vigor manifested among a heterogeneous assortment of seedlings, the respective root-rot and vigor ratings on most of the numbers listed in table 8 have been classified in the double frequency distributions in table 9. Vigor ratings in group A were made in the fall of 1935 and in part repeated in 1936 and in both years were limited to full-grown cane in light soil that permitted only slight to moderate influence of root rot. The latter was judged in various heavy soil plantings. Most of the seedlings of group A and many additional ones, classified in group B, were replanted in the fall of 1936 in heavy soil and root-rot and vigor ratings obtained from the same plots in the spring and fall of 1937, respectively, all estimates being referable to control varieties. In cases of disagreement in ratings between different plots or years, the lowest vigor and the highest root-rot rating were accepted for the purpose of table 9.

TABLE 9.—*Classification of seedling selections according to vigor of growth and susceptibility to root rot*

A. VIGOR RATINGS ON LIGHT, FAVORABLE SOILS AND ROOT-ROT RATINGS ON HEAVY SOIL

Vigor: Class No.	Vigor: Character	Resistant 1	Resistant 2	Intermediate 3	Intermediate 4	Susceptible 5	Total Number	Total Percent
1	Excellent	5	5	6	3	1	20	31.7
2	Good	2	9	5	7	7	30	47.6
3	Fair	1	1	4	3	2	11	17.5
4	Poor	0	0	2	0	0	2	3.2
	Total	8	15	17	13	10	63	
	Percent	12.7	23.8	27.0	20.6	15.9		100.0

B. VIGOR AND ROOT-ROT RATINGS ON HEAVY SOILS

Vigor: Class No.	Vigor: Character	Resistant 1	Resistant 2	Intermediate 3	Intermediate 4	Susceptible 5	Total Number	Total Percent
1	Excellent	14	13	8	1	0	36	34.0
2	Good	4	5	10	6	3	28	26.4
3	Fair	1	8	7	8	4	28	26.4
4	Poor	0	1	2	5	6	14	13.2
	Total	19	27	27	20	13	106	
	Percent	17.9	25.5	25.5	18.9	12.3		100.0

The total number of seedlings is obviously too small for any generalization, but the distribution in group A of table 9 suggests no close association of vigor on light soil with resistance on heavy soil. While 50 percent of the seedlings with "excellent" vigor fall in the resistant classes, the "fair" and "poor" seedlings are not predominantly susceptible. Those of "good" vigor are fairly evenly distributed in all classes. In group B, where both characters were determined on heavy soil and the effect of root rot, plus other unfavorable factors, could directly influence the subsequent vigor ratings, there is a large increase in the percentages of "fair" and "poor" seedlings compared with group A. An association of the two characters (vigor and resistance) is evident from the pronounced trends in the "excellent" and "poor" vigor classes ($r=0.58$, P $0.01=0.25$). However, the "good" and "fair" vigor classes, which comprise more than 50 percent of the seedlings, show, as in group A, a good representation throughout the range of root-rot susceptibility. Therefore, from these limited and somewhat crude comparisons, it appears that vigor per se is not necessarily a controlling factor in resistance except possibly for those seedlings falling in the extreme classes. Thus, in the breeding of improved varieties, only moderate resistance, if accompanied by good vigor, might suffice for light-land use, but a high degree of both qualities must be incorporated for satisfactory yields on the heavy, or more typically root-rot soils.

FIELD INOCULATION TESTS

Reliable quantitative comparison of the degree of resistance or susceptibility of the more important commercial varieties seemed

desirable to supplement and check the visual estimates described on page 71. For this purpose fields were selected that were largely free from *Pythium arrhenomanes*, as indicated by their history and by pot tests with samples of the soil. In two instances they had recently grown three flooded rice crops; in another, five successive legume crops had been plowed under; and in the remainder the land had been abandoned for all cropping for periods of 8 to 10 years. Three to ten varieties were compared in a total of seven replicated plot tests on these areas. The plots of each variety were halved and one part inoculated by scattering pure-culture inoculum of the *Pythium* over the cuttings, with sometimes artificial watering, prior to covering with soil. The fungus was multiplied in large quantity in quart fruit jars on sand-corn-meal medium or corn-meal-extract agar in large Petri dishes.

Examinations during the winter and following spring revealed considerable seed root rot in those tests conducted during 2 years that were particularly favorable for the disease. The *Pythium* was readily reisolated, but in no case did it appear to have spread sufficiently through the soil to affect seriously the growth and yield of plant cane. In two of the tests where sterile inoculum was omitted from the controls, the inoculated plots of nearly all varieties exceeded the latter in yield, due apparently to an indirect fertilizing value of the starchy culture bearing the *Pythium*. Therefore, failure to secure rapid multiplication of the *Pythium* seems most logically due to the biological factors mentioned in the discussion of the influence of soil conditions (pp. 64–67). Therefore, all attempts to obtain a reliable quantitative measure of varietal resistance or susceptibility under natural conditions were frustrated.

In the succeeding stubble crop of most tests, root rot was about as prevalent in the controls as in the inoculated plots, but in neither was it appreciable when compared with that prevalent in old cane land. Nevertheless, in several of the tests the inoculated yielded less than controls, the reductions being greatest in the stubble crop and varying from 1 to 3 tons per acre for Co. 281, C. P. 28/19, C. P. 29/320, and P. O. J. 234. In no instance were they statistically significant.

GREENHOUSE COMPARISONS

Varietal susceptibility tests in soil, especially when conducted in 3- to 4-gallon containers, have proved unsatisfactory and often unreliable, because of difficulty in maintaining a high and uniform moisture content. Such was particularly true for the heavy clay root-rot soil from Louisiana. Comparative seed-root injury has been studied by planting standard-size cuttings in infested soil in deep greenhouse benches. While these data are valuable for checking field ratings of susceptibility, they give no quantitative estimate of disease damage. Therefore, sand-nutrient cultures have been utilized in attempts to measure the particular degree of inherent susceptibility of certain varieties to root rot. These, likewise, have given variable results, especially when conducted at different times of the year, but in general are fairly reproducible when repeated at the same time of year. Even then, they are apparently only of limited value in inter-

preting field performance of a variety. Two experiments on present commercial varieties illustrate this fact and may be summarized for comparison with the field estimates of relative susceptibility or resistance.

EXPERIMENTS 47 AND 62

The experiments on relative susceptibility or resistance of present commercial varieties were conducted in the greenhouse at Arlington Experiment Farm, Arlington, Va. Seed cane of all varieties was of good vigor, free from mosaic, and was produced in a small plot maintained for experimental purposes at Canal Point, Fla. Single-joint cuttings were sprouted in 4-inch pots of steamed silica sand held during the first week in an incubator at 35° C. Later, uniform plants were selected, reset in 3-gallon crocks of steamed sand, inoculated with the moderately virulent isolate No. 58 of *Pythium arrhenomanes* where required, and grown with the aid of nutrient solution D, as described in a previous paper (*80*).

Six of the varieties in experiment 47 were arranged in two 6 by 6 Latin squares, with the inoculated plants and controls of the remaining variety, C. P. 28/19, paired on an adjacent rack; the 6 varieties in experiment 62 were distributed as a randomized block with 10 replications of inoculated plants and controls. The former test was conducted in the early part of the year (February 14 to May 9, 1934) under increasing day length, which promoted rapid growth especially during April, while the latter was run in the fall and winter (September 26, 1934, to January 16, 1935) under minimum illumination. Mean daily temperatures of the sand in both tests ranged between 18° and 25° C., which figures are much below the optimum for cane growth. The experiments were harvested after $3\frac{1}{4}$ and 4 months, respectively, when tillering had reached a maximum, and further growth would represent mainly elongation of existing shoots. However, C. P. 28/19 in test 47 was continued through the summer to determine whether the mean difference between the inoculated plants and the controls, as indicated by the weekly growth measurements, would be eliminated by the higher temperatures and more favorable growing conditions. That such did not occur is in conformity with field experiences.

The results are summarized in table 10, and, except for the very susceptible Louisiana Purple, they show rather wide disagreement in relative damage by the fungus between the two tests conducted during different periods of the year. In experiment 47 there were no significant differences between any of the newer varieties in percentage reduction of green weight, and as a group the damage amounts roughly to only one-third that sustained by Louisiana Purple. Apparently the excellent drainage and satisfactory nutrition prevailing in these sand cultures, combined with good illumination, permitted these more vigorous varieties to counteract largely the damage from root rot. Therefore, to some extent, the results are comparable with the performance of the varieties in light sandy soils in Louisiana.

TABLE 10.—*Comparative root rot susceptibility of commercial varieties in two greenhouse inoculation experiments conducted during different periods of the year*

EXPERIMENT 47 (DATA BASED ON TOTAL OF TWO PLANTS PER CROCK AND SIX REPLICATIONS)

Variety	Initial height		Harvest data													
			Height of primary stalk			Total tillers		Total height (all shoots)				Green weight of tops				
								Actual		Reduction from inoculation		Actual		Reduction [1] from inoculation		
	Controls	Inoculated	Controls	Inoculated	Reduction	Controls	Inoculated	Controls	Inoculated			Controls	Inoculated		
	Centimeters	*Centimeters*	*Centimeters*	*Centimeters*	*Percent*	*Number*	*Number*	*Centimeters*	*Centimeters*	*Centimeters*	*Percent*	*Grams*	*Grams*	*Grams*	*Percent*
Louisiana Purple (control)	10.8	11.6	74.6	59.2	20.6	4.2	2.0	102.2	59.2	43.0	42.1	619.9	396.7	223.2	36.0
Co. 281	15.6	16.8	126.4	126.5	.0	8.5	8.2	193.8	173.8	20.0	10.3	700.8	624.9	75.9	10.8
Co. 290	12.8	11.6	110.8	104.1	6.0	9.8	9.0	186.0	163.0	23.0	12.4	725.5	626.7	98.8	13.6
C. P. 28/19 [2]	17.4	16.2	------	------	------	------	------	897.5	734.2	163.3	18.2	3,167.0	2,762.0	405.0	12.8
C. P. 29/116	20.2	20.2	118.2	115.3	2.4	9.0	6.3	184.0	149.2	34.8	18.9	785.8	698.0	87.8	11.2
C. P. 29/291	18.4	18.8	176.9	170.9	3.4	8.0	6.2	248.2	207.0	41.2	16.6	829.8	690.1	139.7	16.8
C. P. 29/320	6.8	4.8	128.8	114.1	11.4	6.0	5.0	194.4	152.4	42.0	21.6	647.7	522.8	124.9	19.3

EXPERIMENT 62 (DATA BASED ON A SINGLE PLANT PER CROCK AND 10 REPLICATIONS)

Variety	Initial height: Controls	Initial height: Inoculated	Primary stalk: Controls	Primary stalk: Inoculated	Primary stalk: Reduction	Tillers: Controls	Tillers: Inoculated	Total height: Controls	Total height: Inoculated	Reduction from inoculation	Reduction from inoculation	Green weight: Controls	Green weight: Inoculated	Reduction from inoculation	Reduction from inoculation
Louisiana Purple (control)	5.6	4.7	35.3	29.9	15.3	3.5	3.0	83.9	61.3	22.6	26.9	486.6	318.0	168.6	34.6
Co. 281	11.8	11.1	64.2	59.4	7.5	6.7	4.4	154.3	111.2	43.1	27.9	619.7	470.4	149.3	24.1
Co. 290	7.3	7.5	55.5	49.1	11.5	6.1	7.2	169.1	173.0	+3.9	+2.3	828.6	756.9	71.7	8.7
C. P. 807	7.0	7.0	84.0	72.6	13.6	5.0	5.3	159.3	140.9	18.4	11.6	691.8	585.7	106.1	15.3
C. P. 28/19	10.2	9.6	79.0	75.9	3.9	3.5	3.2	146.5	131.0	15.5	10.6	579.5	529.3	50.2	8.7
C. P. 29/320	11.9	13.1	83.7	76.6	8.5	5.2	5.4	180.5	165.2	15.3	8.5	755.8	647.2	108.6	14.4

[1] Reduction required for odds of 19:1 equals 69 g in experiment 47 and 76 g in experiment 62.
[2] Harvested after 7 months, whereas remaining varieties in experiment 47 were harvested after 3¼ months and all varieties in experiment 62 were harvested after 4 months.

Greater differentiation of inherent susceptibility of the varieties is suggested by the results from experiment 62, conducted during the winter months. Here, among the newer varieties, Co. 281 was most susceptible, while C. P. 28/19, which was classed with it in the field tests, displayed resistance equal to Co. 290. However, differences in percentages for the last four varieties in this experiment are likewise not statistically significant.

The data in columns 6 to 15 show that under conditions of poor illumination (experiment 62) the number of tillers ("suckers") of Co. 281 was markedly reduced by the disease, whereas most of the weight reduction of the other varieties was due to smaller size of all shoots and not to a pronounced difference in number. The use

FIGURE 20.—Representative control and inoculated plants illustrating typical differences in severity of root rot between the resistant Co. 290 (*A*) and very susceptible Louisiana Purple (*B*) varieties, photographed after 3 months in experiment 47.

of two plants per crock in experiment 47 resulted in considerable competition for light, so that the number of tillers per plant averaged fewer than those of the second test. Figure 20 shows typical differences in height and number of shoots between control and inoculated plants of Louisiana Purple and Co. 290 in experiment 47.

The data as a whole suggest that tests of this type may be useful for interpreting field performance of varieties, but they are unreliable as a measure of field resistance. For example, the high degree of resistance of the growing plants of C. P. 28/19, shown in experiment 62, emphasizes a particular significance of seed-root damage to this variety in the field. On the basis of this test, it might be expected to suffer no greater damage on root-rot soils than Co. 290, the seed roots of which are rarely seriously rotted. Nevertheless, Co. 290 is placed in the resistant class 1 and C. P. 28/19 in the sus-

ceptible class 5 (p. 72.) Thus, loss of seed roots during unfavorable winter weather is apparently a very serious matter with C. P. 28/19. Seed deterioration troubles following winter root rot, as described in an earlier article (*77*), accentuate the effect of root rot and contribute to its high apparent susceptibility and serious losses in yield on heavy soils. Further investigation may reveal that, due to a predisposing effect for other diseases, only a moderate susceptibility to root rot may have far greater consequences for one variety than for another.

CONTROL OF ROOT ROT

RESISTANT VARIETIES

The investigations summarized in this bulletin emphasize the conclusion of earlier workers that the only generally effective and practical method for controlling root rot is through the introduction or breeding of resistant varieties. The successful application of this method, not only against root rot but for control of mosaic and red rot, is attested by the upward trend of yields in the Louisiana sugar district (fig. 1) following collapse of the industry in 1926.

The writer's studies show that a generally higher degree of resistance to root rot than now obtained will be essential to obtain uniformly satisfactory yields under the frequent extremes of weather and for the large areas of unfavorable soils characteristic of the Louisiana sugar district. The studies show further that highly resistant varieties are necessary to prevent, or at least retard, apparent specialization by the *Pythium* which eventually may bring about declining yields of less resistant varieties. Such specialization or adaptation of this highly variable fungus to a particular variety might be counteracted to some extent by rotating the fields at each replanting with some other equally suitable but genetically different variety. Furthermore, since varieties are known to differ in their requirements of even the ordinary fertilizer elements, rotation of varieties would rest the soil and prevent lowering of yields from possible nutritional idiosyncrasies of any variety succeeding itself repeatedly on the same land. This recommendation, made as early as 1912 by Harrison, Stockdale, and Ward (*50*) in Demerara, has probably been unconsciously followed to a large extent by most Louisiana planters through constant shifting of relative acreages of varieties and increase in area of newly introduced ones.

It is obvious from the above varietal resistance tests that high resistance to root rot is readily secured from interspecific crosses between susceptible noble varieties and the highly resistant wild species (*Saccharum spontaneum*). The great problem of the breeder is, of course, to retain this while combining resistance to other diseases and to introduce, through backcrosses and convergent crosses, the indispensable agronomic and manufacturing qualities required of a commercial variety. Since all elite breeding canes are extremely heterozygous for these characters, the obtaining of a superior resistant variety has thus far depended upon its chance occurrence among large progenies of the many crosses that annually must be produced and tested. Nevertheless, successful continuance of the Department's coordinated breeding (*20*, *87*) and disease-testing program (*76*) should

FIGURE 21.—Comparative stand and development of the root rot-resistant varieties, Co. 290 (*A*) and C. P. 807 (*B*), contrasted with near crop failure of the susceptible C. P. 28/19 (*C*) on poorly drained heavy soil. The differences here shown in September represent a typical sequel of those evident in the spring, illustrated in plate 2, *A*, and figure 18.

result in the discovery of more suitable parental combinations and thus greatly increase the chances of securing superior varieties, without increasing to prohibitive proportions the total number of seedlings to be tested.

The most urgent immediate problem is to find early maturing varieties, combining high resistance to root rot and other diseases and possessing the requisite vigor and yielding capabilities for planting on the mixed and heavy soils. At present the only varieties available for such areas with sufficient vigor and resistance to root rot are Co. 290, C. P. 807, C. P. 29/116, C. P. 28/11, and possibly C. P. 29/320. Figure 21 contrasts the excellent development of two of these varieties with the susceptible C. P. 28/19 on very heavy poorly drained soil. Unfortunately, all of these resistant canes possess one or more serious handicaps, such as late maturity, susceptibility to other diseases, or to storm damage, which make their extensive planting somewhat hazardous and subject to considerable financial losses. Several of the agronomic selections in table 8 and others from more recent seedling series appear promising, on the basis of preliminary tests, for alleviating this situation.

SOIL IMPROVEMENT AND HANDLING

Improvement of soil conditions, particularly drainage and lowering the water table of the heavier lands, the deleterious effects of which are discussed on pages 61–62, not only reduces damage from root rot but increases yields of even the most resistant varieties. Deep preparation by aid of tractors facilitates percolation of water and the penetration of air and roots into the heavier soils that have compacted badly under the rows of the preceding stubble crops.

Great importance is attached to the continuation of experiments on the conservation of cane trash and utilization of factory filter-press cake for improving the physical, biological, and nutritional conditions of old cane lands. Such improvement enables the crop better to withstand injury from root rot. In comparable determinations, 8 tons (fresh weight) of trash and green tops were left in the field from a 21.3-ton-per-acre yield of Co. 281, and 8.8 tons from a 27.7-ton yield of C. P. 807. This material, containing a considerable quantity of nitrogen and high in humus-forming substances, is ordinarily burned, except when the cane stubbles are to be plowed out. Effective and economical incorporation in the soil of that from the preceding crops without detriment to their yields is a challenge to the agricultural engineer and soil-fertility expert. At the average Louisiana sugar factory there is produced about 8 pounds of ashes per ton of cane when all the bagasse is used for fuel, and roughly, 40 pounds of fresh filter-press cake, corresponding to about 20 pounds of dry material per ton of cane. Chemical composition of these byproducts, advantages derived from mixing them, and suggested applications in returning them to the soil have been given by McKaig and Rands (*63*).

Timeliness and thoroughness of all field operations, particularly those connected with planting and getting the crop started off in the spring, are important for promoting vigorous growth which helps the plants of moderately susceptible varieties to replace rotted roots and give fair yields during all but the worst seasons. Careful attention

to all these operations, or, in other words, good farming, brings ample reward from such varieties as Co. 281 and C. P. 28/19, which otherwise may be more or less retarded and reduced in yield by root rot.

SOIL TREATMENTS

Attempts to find direct methods for control of root rot that would not be prohibitive in cost have been unsuccessful. Soil in which the *Pythium* has been killed by chemicals or steam produces good growth of the most susceptible varieties. While such treatments are used to prevent root rot of new seedlings sprouted in the greenhouse, they would, of course, be impracticable under field conditions. However, it was thought that treatment of the soil immediately surrounding the seed cane, in order to prevent winter root rot, might prove commercially feasible. Among nearly a dozen common disinfectants tested in small replicated field plots, Semesan at the rate of 60 pounds per acre was most promising, its effectiveness extending from November through March, after which, however, root rot became nearly as prevalent in the treated areas as in the controls. Nevertheless, total yields of plant cane and succeeding first stubble of the P. O. J. 234 variety were increased by 2.21 tons of cane per acre due to the treatment. The cost of the chemical at this rate of application would be wholly uneconomic.

Since root rot of plant cane on heavy soils is often greatly aggravated by the gnawing and rootlet pruning caused by various kinds of minute soil-inhabiting animals, fumigation experiments were undertaken with the hope of alleviating both troubles. Slowly volatilizing chemicals for protection of the seed roots and young shoot roots through the winter and early spring were tried. In all cases the materials were applied along both sides, and 3 to 4 inches away from the double line of planted cuttings after a small amount of dirt had been scraped over them. Covering of the seed and chemical was then completed with a disk cultivator in the usual manner.

Five fumigants were compared in a replicated plot test with the very sensitive Louisiana Purple variety planted November 14, 1928, in mixed soil. These were orthonitrophenol, paradibrombenzene, paradichlorobenzene, paranitrochlorobenzene, and paratoluidine. Each was applied at the rate of 9.5 g per linear foot of row, or 150 pounds per acre. At the first examination on December 5 root rot was absent in both control and treated plots, due presumably to continuing dry weather and moderately loose condition of the soil. More than 90 percent of the seed roots in control plots were severely pitted near their ends or gnawed off at the time of sprouting, and were generally sparse in development. The paradibrombenzene was by far the best treatment from both standpoints of absence of soil-fauna damage and root toxicity. The cuttings had developed enormous whorls of perfectly sound roots except where they had come too near the chemical that had caused slight injury and a sulphur-yellow color. About half of the substance remained unvaporized. The paradichlorobenzene had completely disappeared, and the roots showed nearly as much soil-fauna damage as the controls. Paranitrochlorobenzene had killed all roots on emergence and many of the eyes, while the paratoluidine was nearly as toxic. However, the latter had

caused great root stimulation on occasional cuttings apparently more removed from its fumes.

Examinations in February revealed no trace or odor of any of the materials; soil-fauna injury and root rot were prevalent in all plots, but strikingly less so in those treated with the paradibrombenzene, which apparently had exerted some fungicidal as well as an insecticidal effect. When harvested November 9, 1929, the 10 plots of this treatment averaged 33.1 tons of cane per acre compared with 28.7 tons from the controls, or a significant increase of 4.4 tons due to the treatment. The paranitrochlorobenzene reduced yields by 9 tons per acre, and the remaining chemicals had no significant influence. The effect of the above treatments on the population of the minute soil animals responsible for the root gnawing was studied by J. W. Ingram, of the Bureau of Entomology and Plant Quarantine, United States Department of Agriculture, and reported on by him (*55*).

Paradibrombenzene and paradichlorobenzene were tested during 1929–30 in several black-land experiments on Co. 281, C. P. 807, and P. O. J. 36–M. One-fourth, one-half, and the full rate of application used in the previous test were compared. Winter rains of 1929–30 compacted the surface soil, which apparently so confined the fumes that considerable burning of roots and reduction in germination were caused by all but the weakest treatment. The latter showed no observable influence on either soil-fauna injury or root rot. A pronounced odor of the chemicals in soil from the stronger treatments could be detected as late as April 21. Average yields in November revealed differences, depending apparently upon soil conditions, ranging from a 2-ton gain for Co. 281 to an 8-ton loss in the case of C. P. 807, due to treatment. Therefore, on the basis of these preliminary tests it appeared that even if an economical and effective rate of application should be found, its utilization in some years would be attended by sufficient hazard to render it unsafe for commercial use.

SUMMARY

Since the failure in 1923 to 1926 of the old noble varieties of sugarcane in Louisiana, which was due to combined damage from mosaic, red rot, and the so-called root disease, the last-mentioned trouble has continued to be a serious problem; this despite the restoration of the industry from the introduction of somewhat more resistant and vigorous hybrid canes. On the latter, root rot is obviously the most important factor in the root-disease complex, even in exceptional cases when the symptoms approach those characteristic of the condition on the old varieties.

An apparent increase in the severity of root rot on certain of the newer varieties following their widespread and continued cultivation has emphasized the need for fundamental knowledge about the disease as a necessary basis for determining the resistance of new seedling selections and securing and maintaining further yield improvement.

Root rot was noted to be causing widespread damage in Louisiana as early as 1908, when red rot of the seed cane was also reported. Combined damage from the two diseases during the period 1910–20 reduced State-wide average yields by 23 percent, and the subsequent

mosaic epidemic another 30 percent, which brought the industry to virtual bankruptcy. Varietal introductions by the Department and cooperating agencies (Louisiana Agricultural Experiment Station and the American Sugar Cane League) have gradually restored production to approximately that of the earlier long-period level of 1888–1907.

On certain of the presently grown and moderately susceptible varieties, such as Co. 281 and C. P. 28/19, root rot is usually manifested merely by unthrifty appearance, deficient and delayed tillering (suckering), and closing in of the rows. During occasional bad root rot years yellowing of the leaves, severe wilting, and death of young plants may result in poor stands and virtual crop failure on heavy clay soils, due to complete destruction of roots on both seed cuttings and young shoots.

As indicated in preliminary reports, *Pythium arrhenomanes* Drechsler was found to be the principal cause of the root rot. Although during the past quarter century root-disease epidemics have been reported from most sugarcane-producing countries, this particular fungus has been identified only from Hawaii, the Philippine Islands, Mauritius, Canada (where it attacks cereals), and the United States.

Twelve additional species of *Pythium* and several other fungi were isolated from decaying roots obtained in surveys of the sugar- and sirup-producing sections of the Gulf States. They were most numerous in Louisiana where the roots had either been injured by the gnawing of minute soil fauna or were weakened by red rot of the cuttings or some unfavorable soil condition. Infection tests conducted under a wide range of environmental conditions in the greenhouse were negative to the extent of development of a general root rot characteristic of *P. arrhenomanes*. They also showed no tendency to act as secondary invaders to the latter fungus. However, under the predisposing influence of dilute concentrations of a soil toxin (salicylic aldehyde) severe root rot and appreciable reduction of plant weight were caused by several of these miscellaneous species, particularly *P. dissotocum* and *P. graminicolum*. These results in conjunction with the survey records suggest that only under very abnormal conditions may any of these species contribute to an important extent in the destruction of sugarcane roots.

Physiologic specialization and, to some extent, varietal adaptation of *Pythium arrhenomanes* in the Louisiana sugar district have been revealed by greenhouse inoculation experiments with more than 200 isolates of this species obtained in root rot surveys of representative plantations. Significant differences in average virulence of the isolates were found to occur between different plantations or localities, as well as between an earlier (1927–31) and a more recent (1935–36) survey.

Since the latter finding could not readily be explained on the basis of attenuation, resulting from prolonged maintenance in artificial culture of the early collection, actual increase in average virulence of the fungus during the period of 5 to 7 years separating the surveys is tentatively assumed to have occurred. This is conceivably due in part at least to segregation and multiplication of certain biotypes brought about by general adoption of more resistant varie-

ties, which permitted survival of only the more virulent or adaptable components of the earlier population of the fungus.

A serious decline in yield of the susceptible P. O. J. 234 in relation to the highly resistant Co. 290 and C. P. 807 varieties in replicated agronomic yield comparisons during the past 8 years has been associated with apparent increase in root rot severity, and possibly reflects in part at least the above-found increased virulence of the *Pythium*.

No increase in root rot of resistant varieties has been observed, although one isolate of the *Pythium* was found capable of seriously damaging the Co. 290 in greenhouse tests. However, this may represent merely a chance variant rather than a specialized subpopulation of more virulent forms in the fields.

Physiologic specialization of *Pythium arrhenomanes* and its potential influence on yields show that root rot must be looked upon as a dynamic rather than a static factor, as hitherto considered in relation to sugarcane production. Therefore root rot-resistance tests of new seedling selections necessitate prior artificial infestation of the soil with a collection of the most virulent locally known cultures of the fungus.

The apparent degree of resistance or susceptibility in field tests of well-known varieties, representing the recognized species of sugarcane, is given. Most noble varieties (*Saccharum officinarum*) were found to be highly susceptible, while the Chinese canes (*S. sinense*) and the wild sugarcane (*S. spontaneum*) were highly resistant. Two Indian varieties of *S. barberi* occupied an intermediate position.

F_1 hybrids from crosses between the susceptible, noble, and resistant wild cane were usually resistant, but successive backcrossing to the noble parent ("nobilization") to secure commercial qualities gave increasing susceptibility in the few seedlings studied.

Since most elite breeding canes possess extremely complex inheritance, an important object of the Department's coordinated sugarcane breeding and disease-testing program is to discover more suitable parental combinations that will increase the chances of securing superior resistant varieties without increasing to prohibitive proportions the total number of seedlings to be tested.

Tentative root rot ratings on 111 first-year or later agronomic selections revealed nearly one-half to be resistant. If one or more of these should be found to combine the indispensable other qualities, especially early maturity, hazards from use of the presently available root rot-resistant varieties may be greatly minimized.

Among the present commercial varieties in Louisiana, Co. 290, C. P. 807, C. P. 28/11, and C. P. 29/116 are classed as resistant to root rot and also possess sufficient vigor for planting on the mixed and heavy soils. However, plantings of C. P. 807 have already been greatly diminished because of too great susceptibility to red rot. C. P. 29/320 has not been seriously damaged by root rot, but has been reported to be susceptible to red rot. Co. 281 and C. P. 28/19 are susceptible to root rot and ordinarily succeed only in light, well-drained soils.

Detailed studies confirmed the conclusions of other investigators that high winter rainfall and low spring temperatures greatly accentuate the damage to fall-planted cane. Summer planting of C. P. 28/19, when it must be grown on heavy soils, was found to prevent the serious losses in yield and the practical crop failure sometimes experienced with regular October plantings.

In controlled soil temperature tanks root rot was worst at 65° to 68° F., and became progressively less serious with increase in temperature to 97° which is past optimum for cane growth. The effect of a more virulent strain of the *Pythium* was characterized by greater damage, particularly at intermediate and high temperatures, while the use of a more resistant variety tended to suppress the disease, particularly at these temperatures.

Greater severity of root rot on mixed and heavy clay root-rot soils emphasize the need for better drainage, deep preparation by tractors, and other measures to prevent practical waterlogging during periods of prolonged rainfall. The greatly accentuating effect on root rot of toxic materials possibly accumulating under such deficient aeration has been indicated by greenhouse experiments.

Increased root rot of plant cane has been noted to result apparently from excessive nitrogen fertilization of the crop furnishing the seed.

The comparative unimportance of root rot on muck and peat soils is apparently ascribable (among other things) to their greater biological activity and possible antibiotic effect on spread of the *Pythium*.

Improvement of the physical, chemical, and biological conditions of the root rot soils by continued plowing under of all cane trash and by moderate applications of factory filter-press cake or stable manure, when also accompanied by good drainage, has markedly reduced root-rot damage of susceptible varieties.

Attempts to discover a soil treatment or other direct methods for control of root rot that would not be prohibitive in cost have been unsuccessful.

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